Appendix 1: Supplementary figures

Supplementary figures about the statistical analysis

The figures from A1 to A4 below show some extra output of the statistical analysis the diagnostic plots of the standard regressions and the log-likelihood function of the phylogenetic regression.

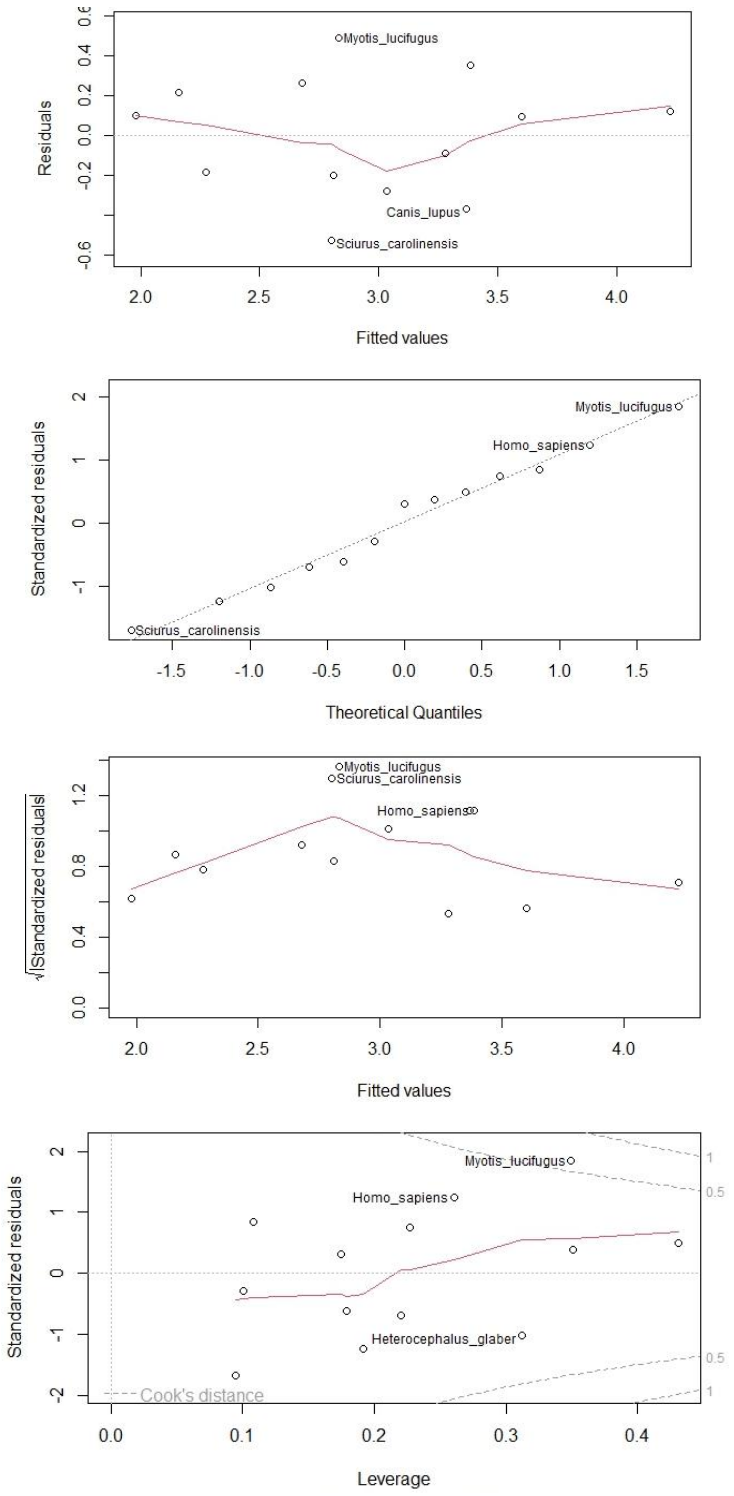


Figure A1: Diagnostic plots of the MLR model "ln(PD) vs ln(mass) + BMRr". The plots represent: a) Residuals vs Fitted values, b) Normal quantile-quantile plot, c) Scale-location plot, d) Residuals vs Leverage. Myotis lucifugus, Sciurus carolinensis and Homo sapiens show slightly higher standardized residuals than expected (in absolute value), but no major departure from the assumptions is noted.

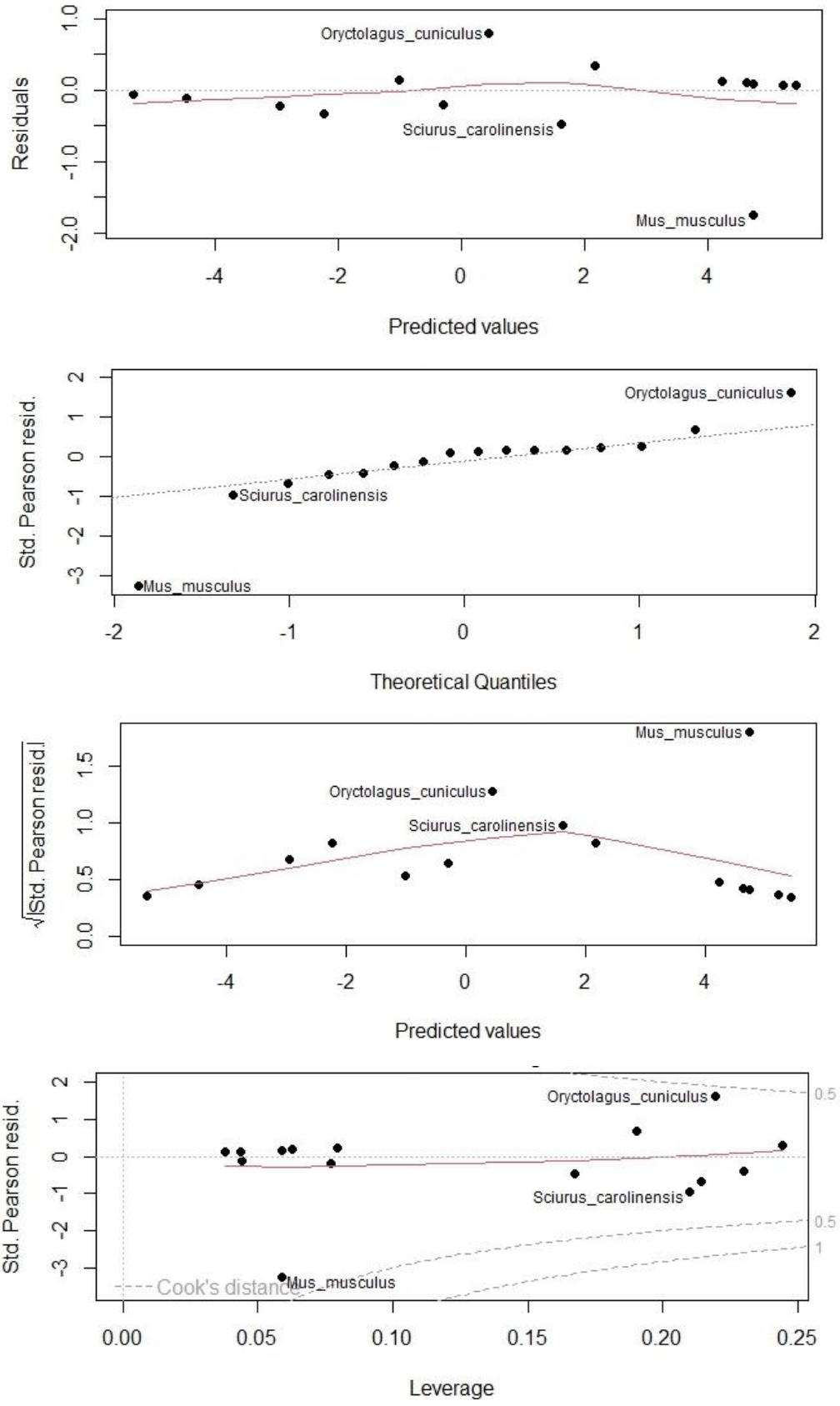


Figure A2: Diagnostic plots of the MLR model "IF vs ln(mass)". The plots represent: a) Residuals vs Fitted values, b) Normal quantile-quantile plot, c) Scale-location plot, d) Residuals vs Leverage. Mus musculus has a very large standardized residual, and might be an influential variable. Also, Oryctolagus cuniculus and Sciurus carolinensis show some potentially critical values.

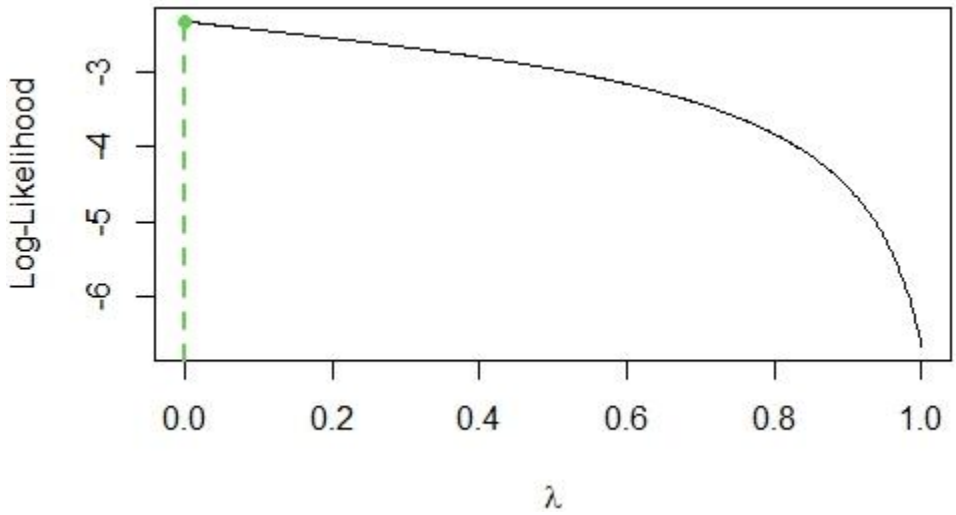


Figure A3: Log-likelihood function of the phylogenetic model ln(mass) vs ln(PD) for values of Pagel λ ranging between 0 and 1.

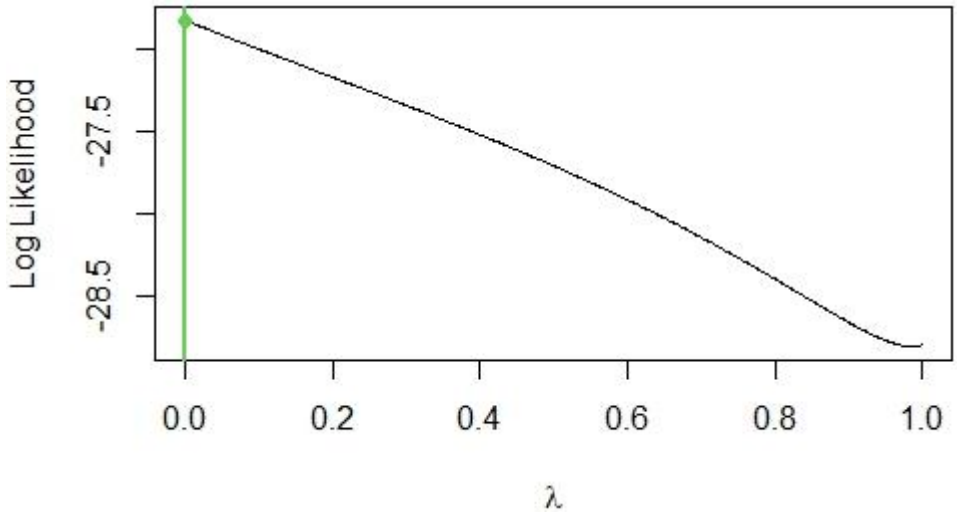


Figure A4: Log-likelihood function of the phylogenetic model ln(mass) vs logit(IF) for values of Pagel λ ranging between 0 and 1.

Details about the cultured cell strains

The table below provides, for each strain cultivated in the study, the information about donor's age, PD at phase out, maximum observed PD, and immortalization.

31 strains (of *rattus norvegicus*, *macaca mulatta*, *homo sapiens*, *gorilla gorilla*, *equus ferus caballus*, and *bos taurus*) were obtained from the Coriell Institute for Medical Research (CIMR, Camden NJ, USA). The CIMR procedure, for cultures initiated from explants, is to assign PD = 0 to cells of the primary culture at confluency. We have established, with the method proposed by Pignolo et al. [20], six independent human lines and calculated that, on average, a primary culture of human fibroblasts derived from skin explants accomplishes 7.5 PD before reaching confluence in a T25 flask. Accordingly, we corrected the PD count for the cultures obtained from Coriell Institute by 7.5. In the plots of this section, it is possible to identify the strains coming from the Coriell Institute by their code, which starts with "AG".

23 strains were coming from previously published studies, specifically:

- 5 strains from 2 6-months-old Norway rats, with average PD=19 [20];
- 18 strains from 11 young/adult humans, with average PD=40.95 (standard deviation=6.12) [21].

These 23 strains, although included in our present analysis, are not shown in the table below. The table presents all the strains for which here we report cumulative growth curve.

Species		**Donor**		**Strain**			
Code	**Scientific name (Common name)**	**Code**	**Age at sampling**	**Code**	**Phased out**	**Max PD obs.**	**Immort. obs.**
LBB	Myotis lucifugus (Little brown bat)	#1	<3 y.o. (estimated)	LBB#1	\	66.94	Yes
				LBB#2	\	70.23	Yes
		#2	<3 y.o. (estimated)	LBB#3A	\	101.6	Yes
				LBB#4A	\	100.6[a]	Yes
				LBB#5A	27.86	69.27	Yes
				LBB#6A	\	103.53	Yes
				LBB#7A	\	105.81	Yes
TB	Tadarida brasiliensis (Mexican free tailed bat)	#1	3 y.o. (estimated)	TB#1	\	76.55	Yes
				TB#2	\	106.75	Yes
MM	Mus musculus (Mouse)	#1	3 m.o. (estimated)	IHM#BA	\	24.21	Yes
				IHM#BB	\	25.90	Yes
		#2	2 m.o.	BM#1	11.17	11.17	No
				BM#2	\	24.36	Yes
				BM#3	6.81	7.36	No
				BM#6	5.20	5.74	No
				BM#7	11.60	28.66	Yes
				BM#8	9.12	22.17	Yes
		#3	3 m.o. (estimated)	PHM#2	9.94	21.18	Yes
				PHM#3	4.97	19.66	Yes
				PHM#4	6.75	14.87	Yes
PM	Peromyscus maniculatus (Deer mouse)	#1	Young/adult (estimated)	PMS#B	7.32	17.42	Yes
		#2		PMS#R	20.00	25.82	Yes
		#3		PMS#L	11.59	16.54	Yes
		#1	4 m.o.	PMBF#B	9.75	14.08	Yes
		#2		PMBF#R	7.59	15.29	Yes
		#3		PMBF#L	8.23	27.71	Yes
BBB	Eptesicus fuscus	#1	<3 y.o.	BBB#1	15.50	21.86	Yes

	(Big brown bat)		(estimated)				
		#2	<3 y.o. (estimated)	BBB#2	11.71	20.29	Yes
PL	Peromyscus leucopus (White-footed mouse)	#1	4 m.o.	PL#B	9.93	11.16[b]	\
				PL#R	8.71	17.61	Yes
				PL#L	5.61	32.52	Yes
N	Heterocephalus glaber (Naked mole-rat)	#1	1 y.o.	N#32	15.72	19.05	Yes[c]
				N#B[d]	10.60	10.83	No
				N#L[d]	7.90	11.78	No
RN	Rattus norvegicus (Norway rat)	#1	6 m.o.	R#Y1	\	24.68	Yes
		#2	6 m.o.	R#Y2	\	26.92	Yes
		#3	6 m.o.	R#Y3	\	27.57	Yes
		#4	12 m.o.	R#A5	\	22.46	Yes
		#5	12 m.o.	R#A6	\	22.39	Yes
		#6	18 m.o.	R#O7	\	27.79	Yes
		#7	18 m.o.	R#O8	\	26.43	Yes
		#8	18 m.o.	R#O9	\	28.10	Yes
S	Sciurus carolinensis (Eastern gray squirrel)	#1	2.5 y.o. (estimated)	S#1	7.39	9.81	No
				S#2	11.93	15.93	Yes
				S#3	9.87	22.86	Yes
RA	Oryctolagus cuniculus (Rabbit)	#1	3.5 m.o.	RA#1	\	58.73	Yes
				RA#2	\	61.86	Yes
				RA#3	\	74.91	Yes
		#2	3.5 m.o.	RA#4	\	75.91	Yes
				RA#5	\	68.37	Yes
FC	Felis catus (Domestic cat - tabby)	#1	3 y.o.	C3#L	\	60.82	Yes
				C3#L2	\	38.67	Yes
		#2	Young/adult (estimated)	CN#1	34.55	34.55	No
				CN#2	28.10	28.10	No
		#3	12 y.o.	C12#BA	21.87	22.47	No
				C12#BB	12.65	13.67	No
RM	Macaca mulatta (Rhesus monkey)	#1	2 y.o.	AG07107	19.21	49.00	Yes
		#2	5 y.o.	AG06252	31.03	32.08	No
		#3	5 y.o.	AG07127	70.57	70.78	No
D	Canis lupus familiaris (Dog - Beagle)	#1	13 m.o.	D#1	35.24	36.03	No
				D#2	17.41	17.71	No
				D#3	24.80	24.80	No
				D#4	42.14	42.16	No
D	Canis lupus familiaris (Dog - Rottweiler)	#2	Young/adult (estimated)	RW#1	17.53	17.53	No
				RW#2	10.69	10.74	No
				RW#3	10.33	10.33	No
				RW#4	8.60	8.60	No
		#3	Young/adult (estimated)	RW2#1	8.90	8.90	No
H	Homo sapiens (Human)	#1	29 y.o.	H#1	31.82	41.88	No
				H#2	39.24	49.08	No

				H#3	37.22	38.46	No
				H#4	43.86	52.01	No
		#2	30-40 y.o.	H2#1	70.02	70.68	No
				H2#2	47.53	58.75	No
G	Gorilla gorilla (Low land gorilla)	#1	4 y.o.	AG05251	81.37	81.37[e]	No
EQ	Equus ferus caballus (Horse)	#1	2 y.o.	AG08029	59.56	59.78	No
		#2	3 y.o.	AG07906	38.77	38.77	No
		#3	6 y.o.	AG08032	47.09	47.09	No
B	Bos taurus (Cattle - Holstein)	#1	15 w.o.	AG10466	80.71	86.58	No
		#2	5 m.o.	B#1	77.63	78.37	No
				B#2	70.45	71.48	No
				B#3	78.70	78.84	No
				B#4	69.65	70.67	No

Table A2: Summary of all the strains cultivated in the study.

[a] The strain was kept being cultivated afterwards, reaching PD = 170.22.

[b] The strain culture was stopped due to contamination. Hence, it was impossible to assess immortalization.

[c] The estimate of the immortalization probability (100%) was considered in the analysis even if it was based on just one strain, as this value is confirmed by the scientific literature [36].

[d] The strains were cultivated at 37°C, instead of 32°C (body temperature of naked mole-rat in vivo). For this reason, they were excluded from the statistical analysis.

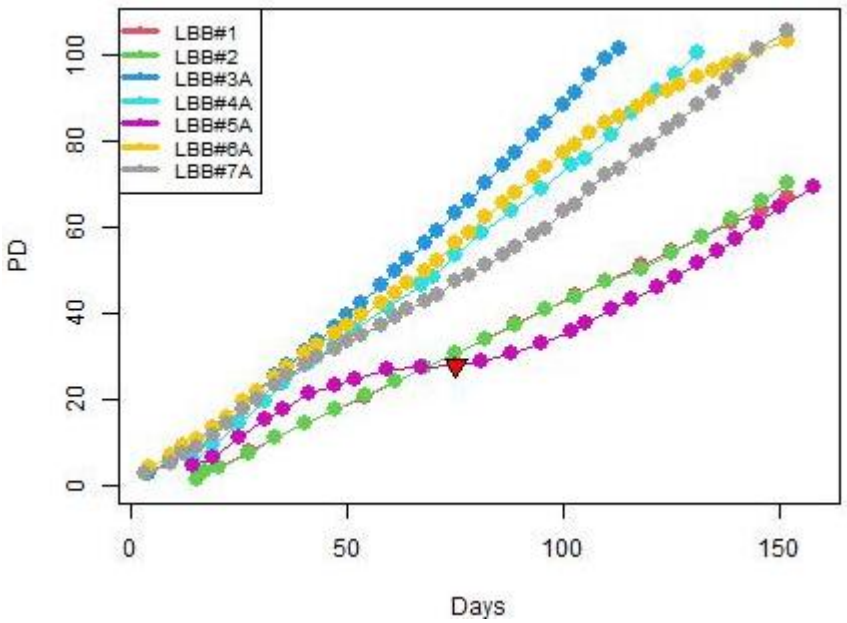
[e] The estimate of the immortalization probability (0%) was not considered in the analysis, as it was based on just one strain.

The following pages contain a summary sheet for each species analyzed. Each sheet includes:

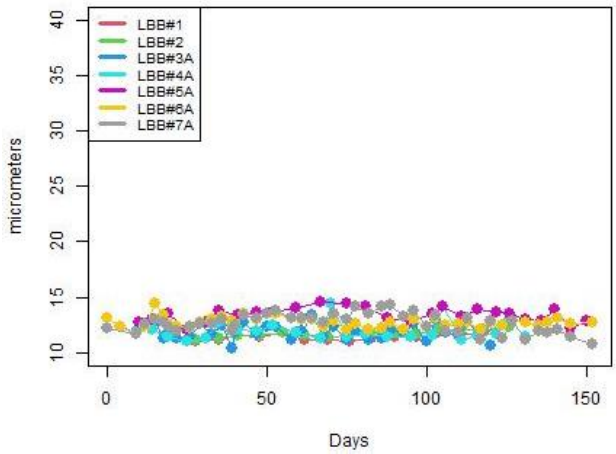
- scientific name and common name of the species
- a representative picture of the species
- cumulative growth curves of the strains,
- average cell diameter curves,
- histogram(s) about the chromosome number distribution with the indication of the normal 2n number of chromosomes
- phase contrast micrographs of the cells taken at different days of culture.

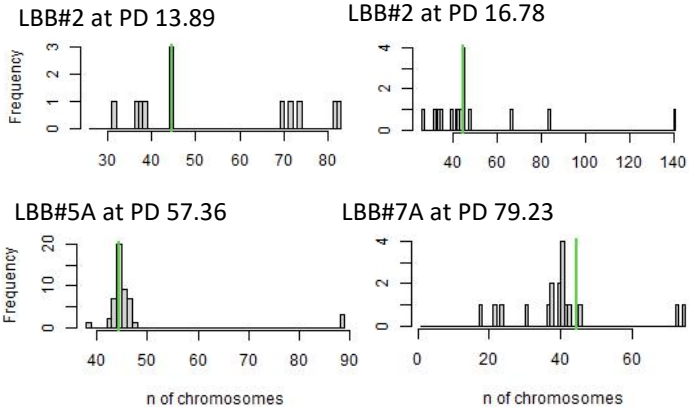
The 23 strains taken from other publications [20,21] are excluded from the summary. The species are sorted ascendingly by average body mass.

Mtyotis lucifugus (Little brown bat)

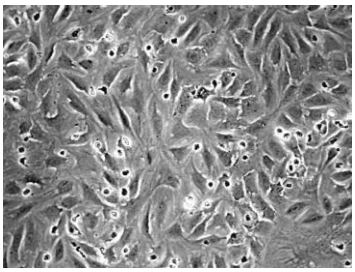


2n=44

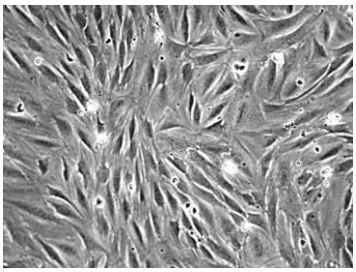




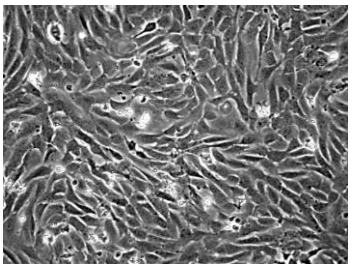
LBB#2



Day 35 - 20X Objective

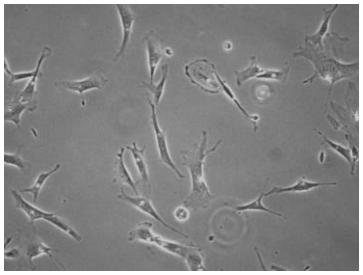


Day 83 - 20X Objective

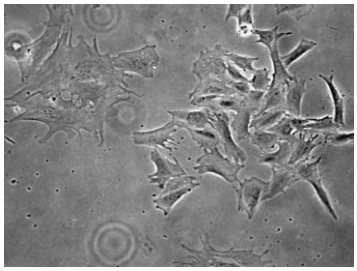


Day 159 - 20X Objective

LBB#5A

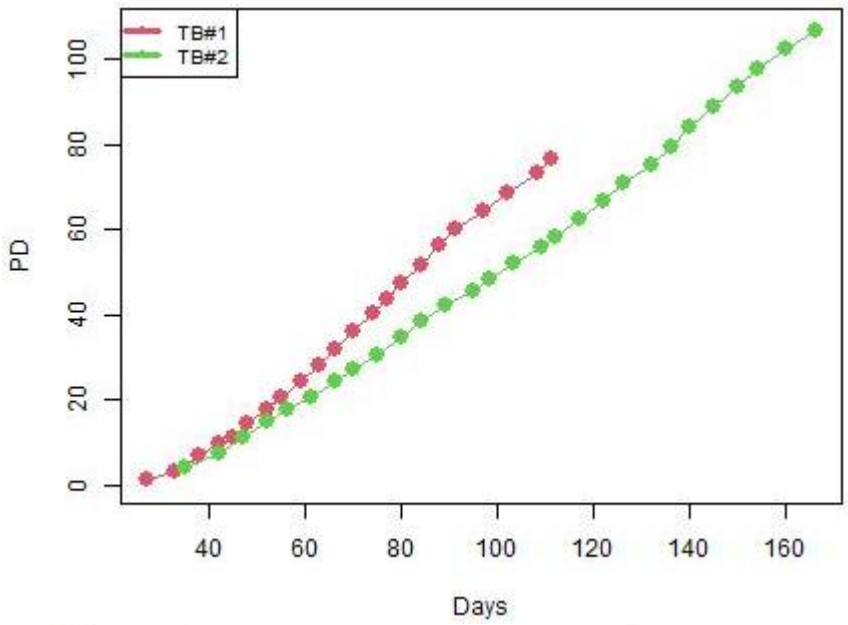


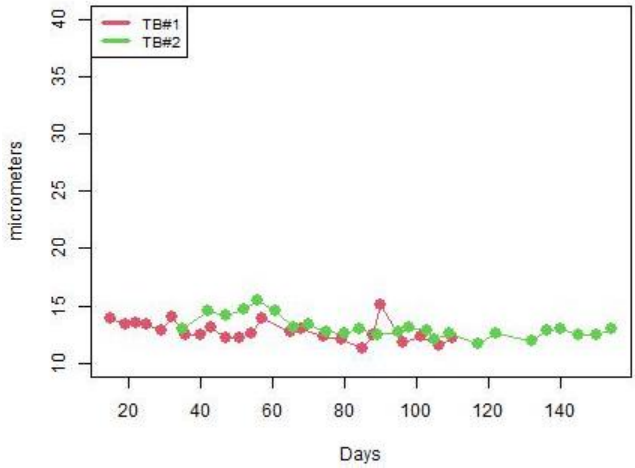
Day 17 - 20X Objective



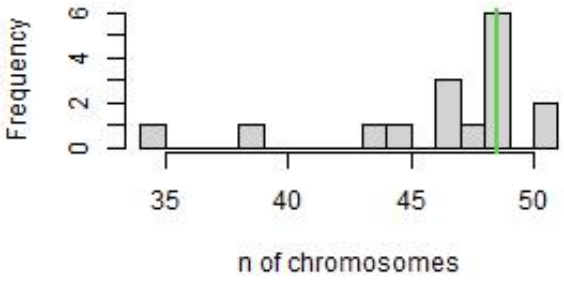
Day 74 - 20X Objective

Tadarida brasiliensis (Mexican free tailed bat)

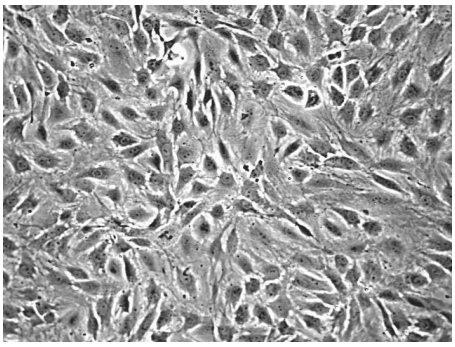




TB#1 at PD 31.82 (2n=48)

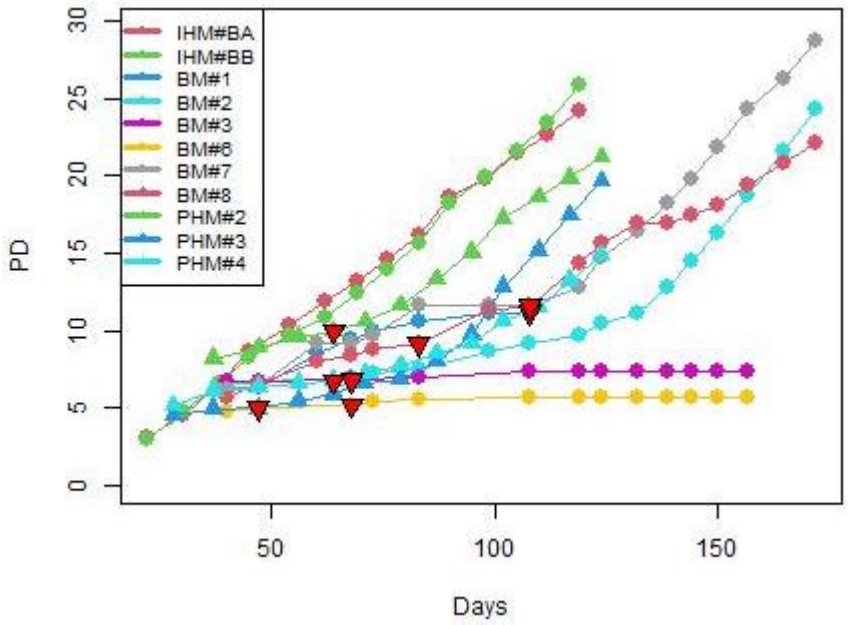


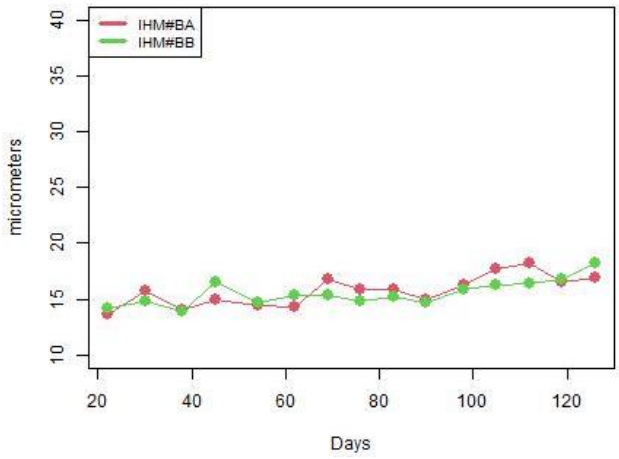
TB#1



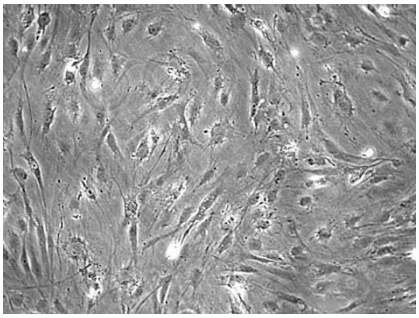
Day 19 - 20X Objective

Mus musculus (House mouse)



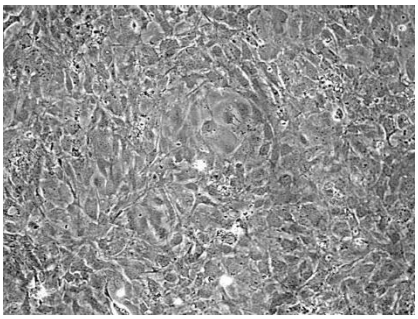


IHM#BA



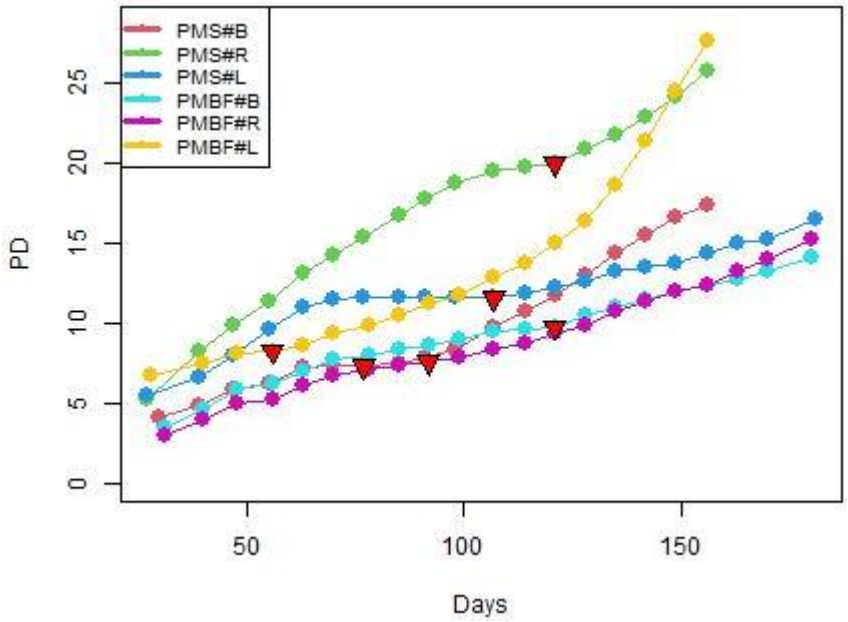
Day 126 - 10X Objective

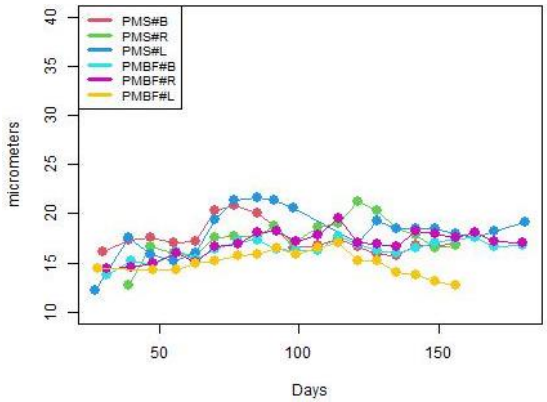
IHM#BB



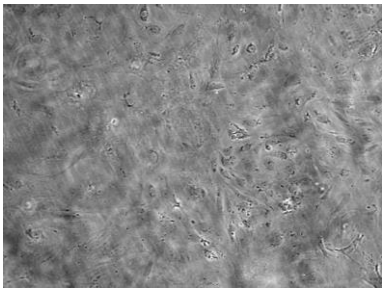
Day 126 - 10X Objective

Peromyscus maniculatus (Deer mouse)

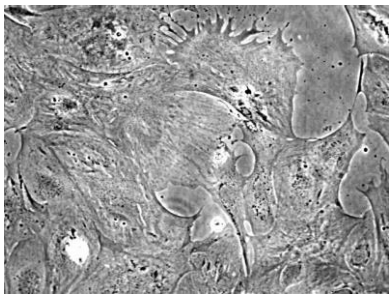




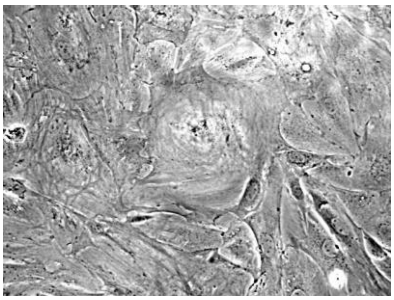
PMS#L



Day 119 - 10X Objective

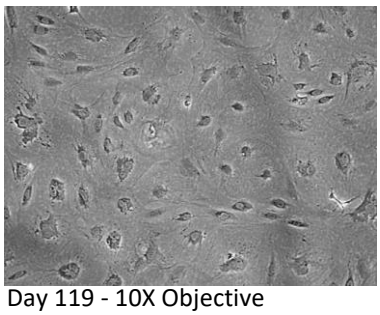


Day 140 - 20X Objective

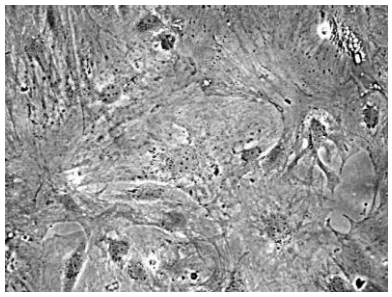


Day 179 - 20X Objective

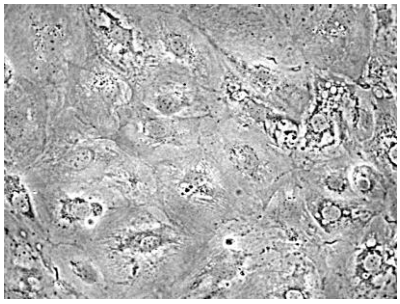
PMBF#B



Day 119 - 10X Objective

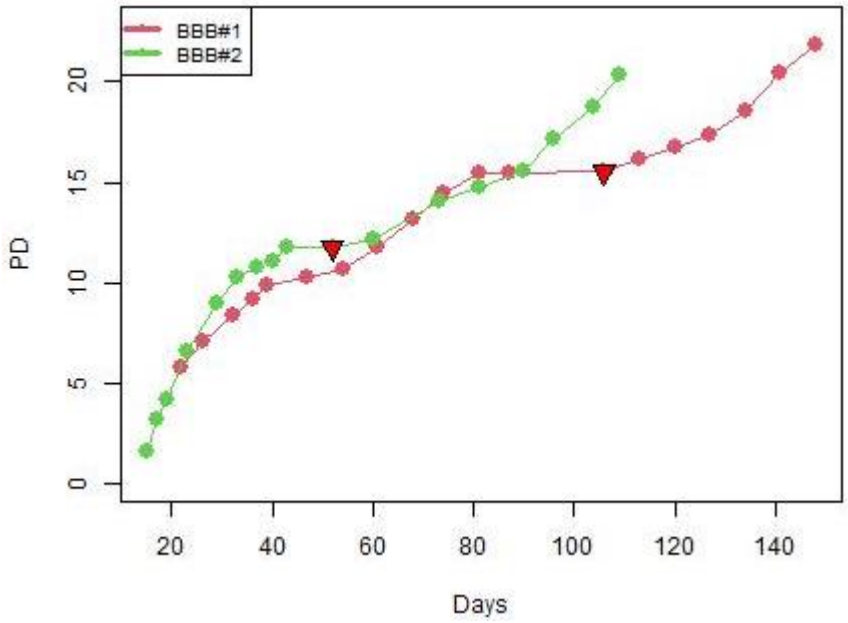


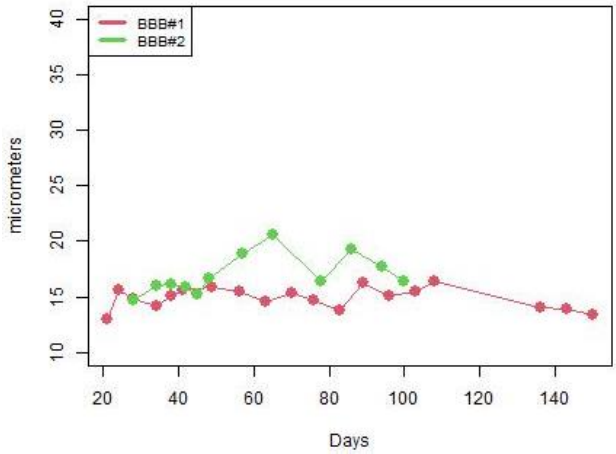
Day 140 - 20X Objective



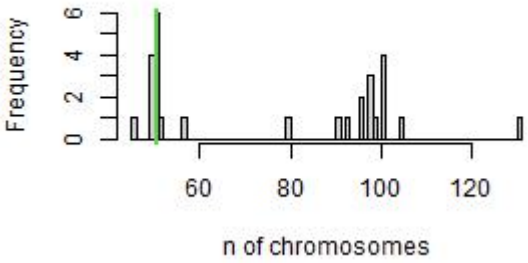
Day 178 - 20X Objective

Eptesicus fuscus (Big brown bat)

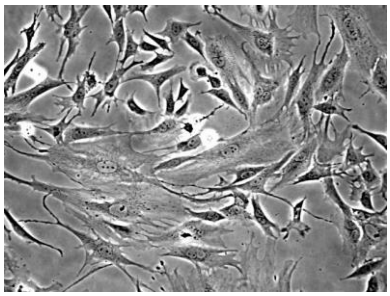




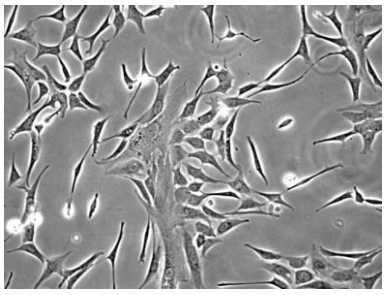
BBB#1 at PD 5.73 (2n=50)



BBB#1

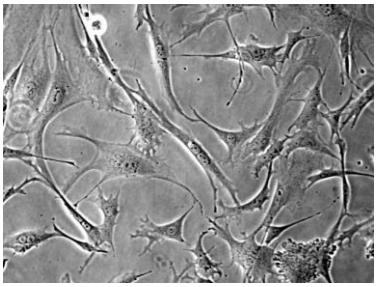


Day 28 - 20X Objective

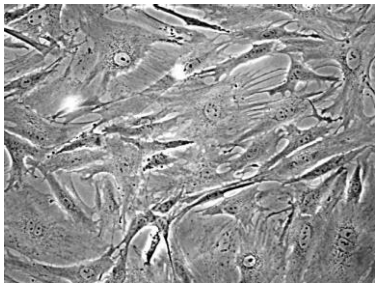


Day 151 - 20X Objective

BBB#2

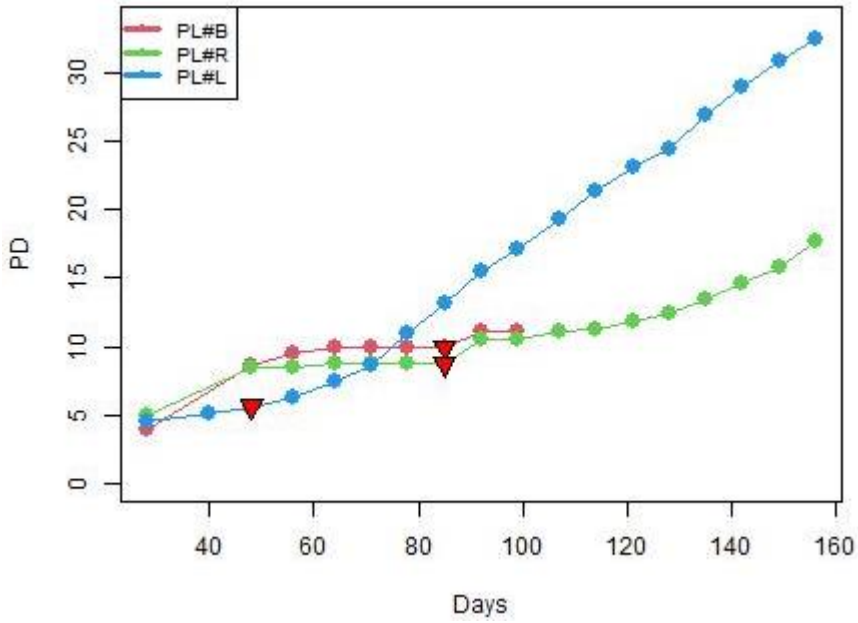


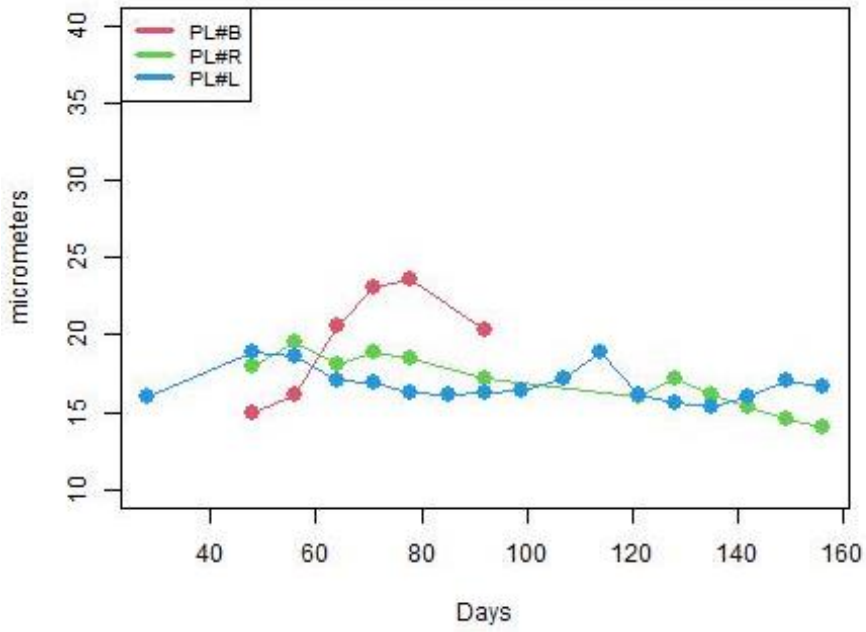
Day 42 - 20X Objective



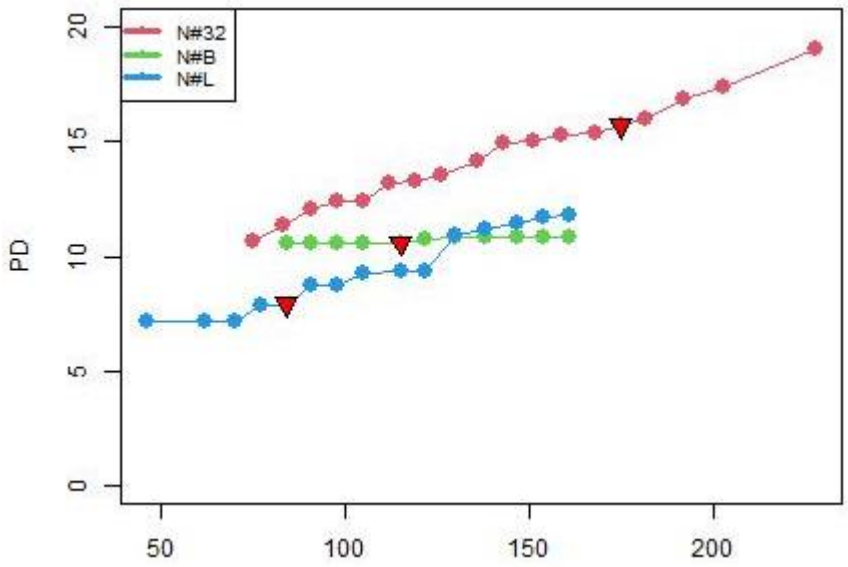
Day 48 - 20X Objective

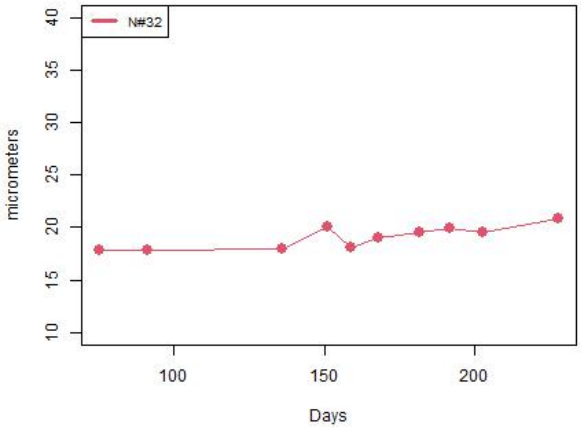
Peromyscus leocopus (White-footed mouse)



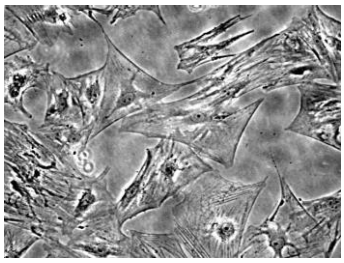


Heterocephalus glaber (Naked mole-rat)

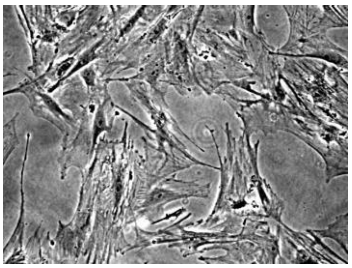




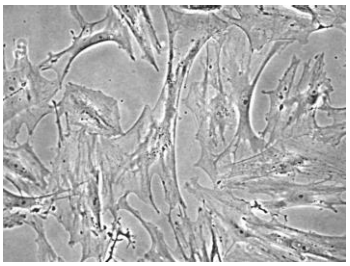
N#32



Day 159 - 20X Objective

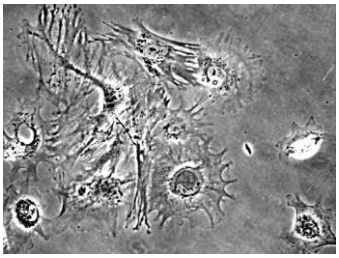


Day 182 - 20X Objective

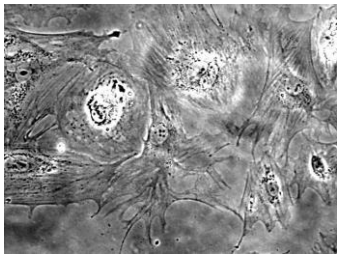


Day 208 - 20X Objective

N#B

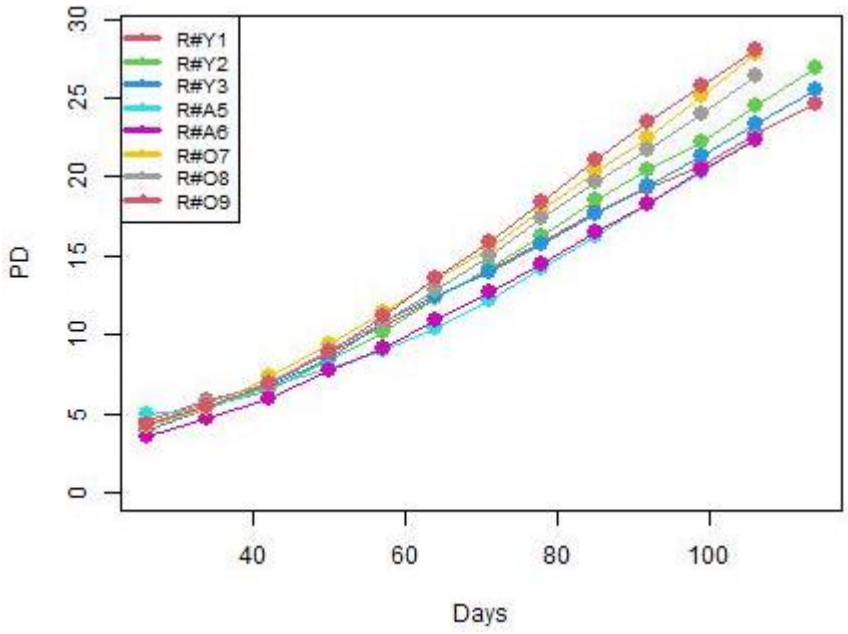


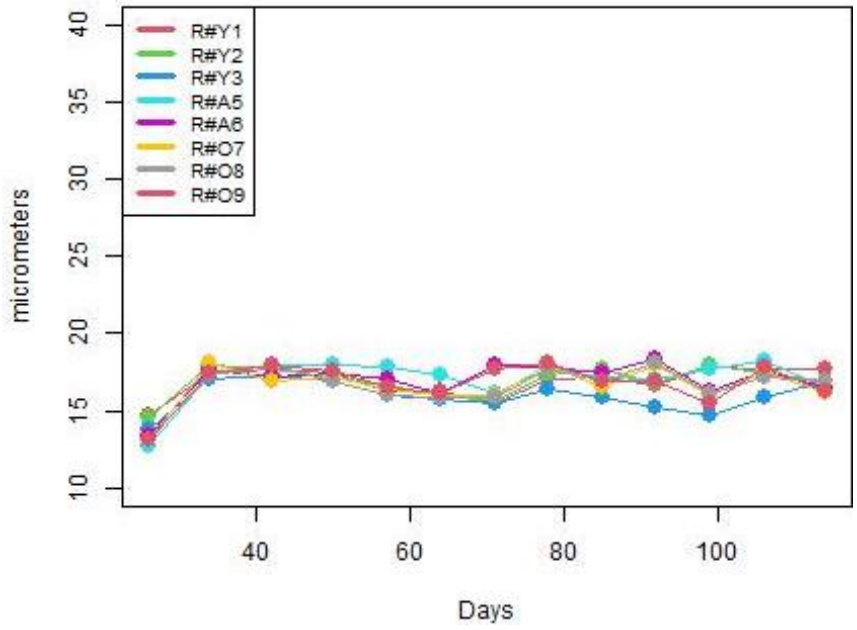
Day 138 - 20X Objective



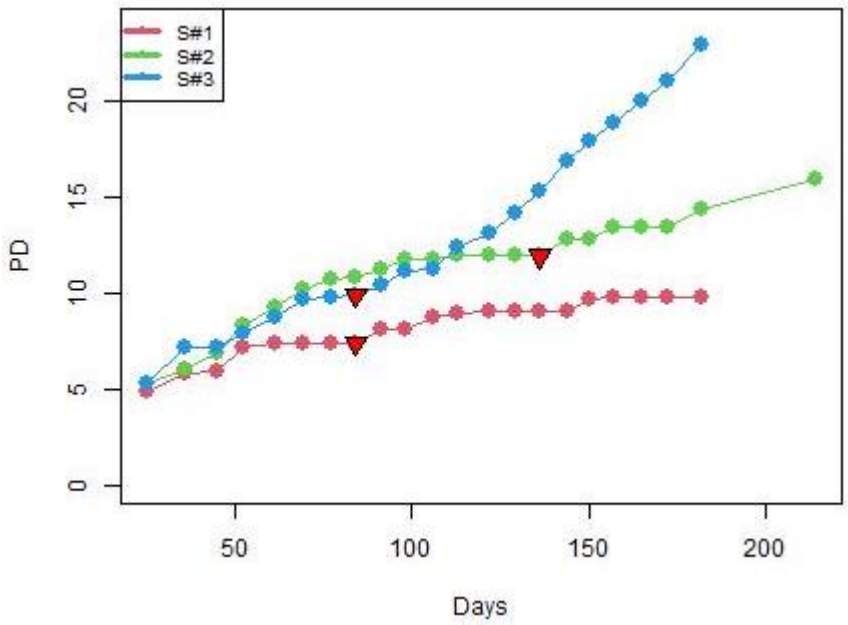
Day 161 - 20X Objective

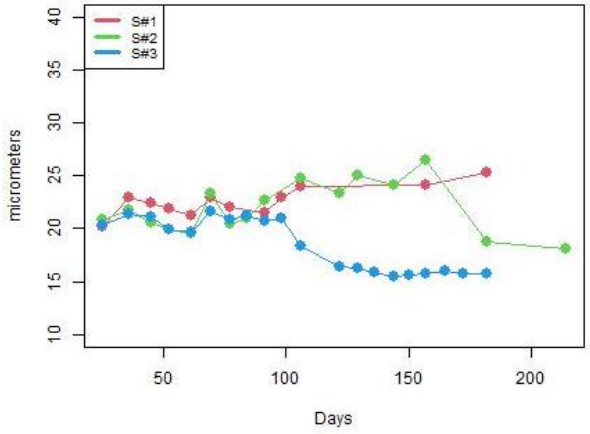
Rattus norvegicus (Norway rat)



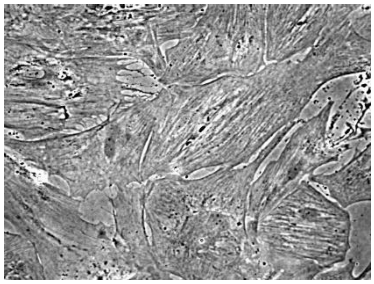


Sciurus carolinensis (Squirrel)

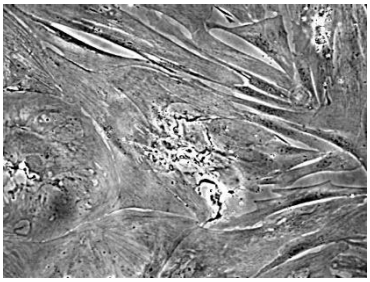




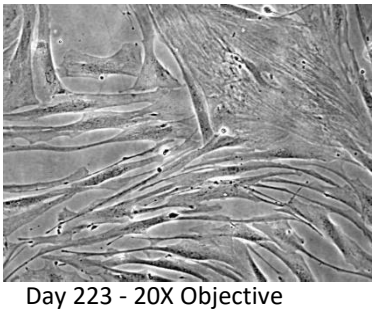
S#2



Day 144 - 20X Objective

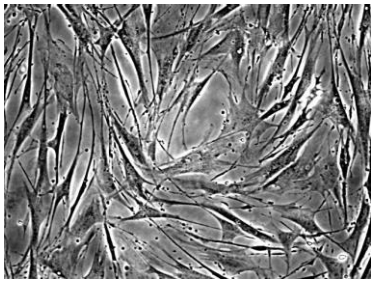


Day 182 - 20X Objective

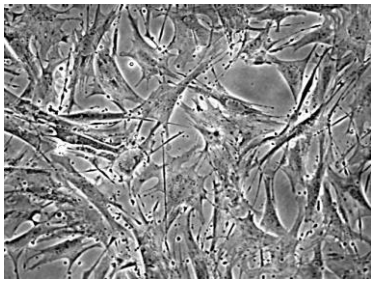


Day 223 - 20X Objective

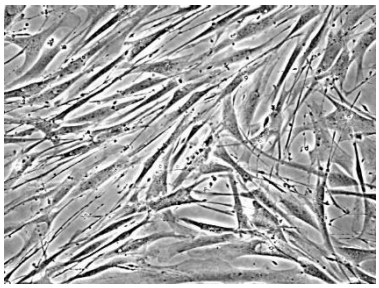
S#3



Day 144 - 20X Objective

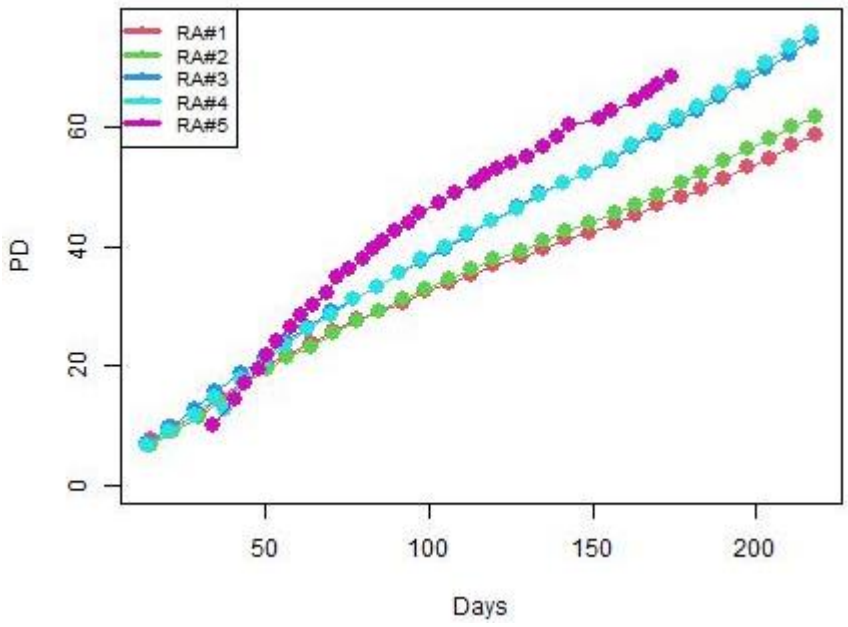


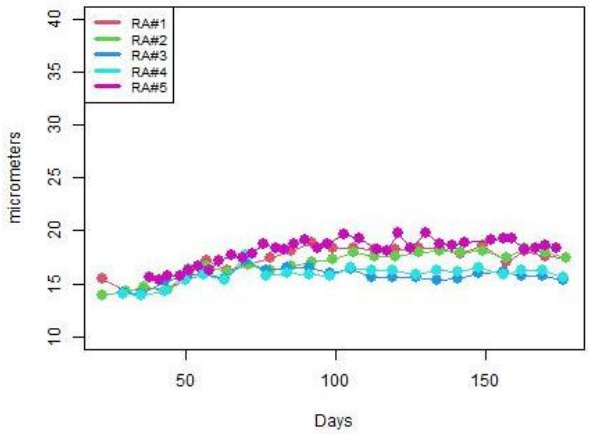
Day 157 - 20X Objective



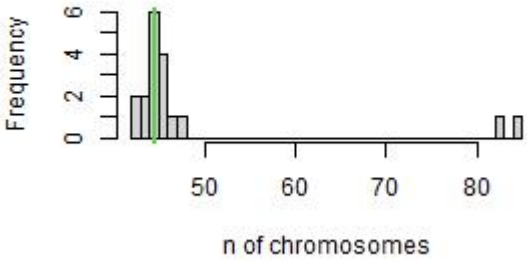
Day 185 - 20X Objective

Oryctolagus cuniculus (Rabbit)

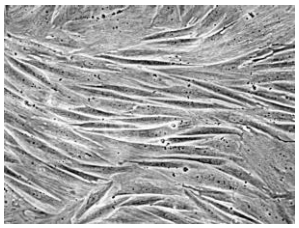




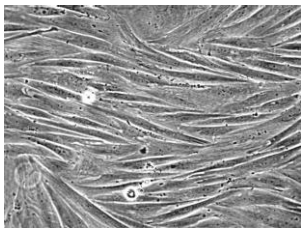
RA#5 at PD=28 (2n=44)



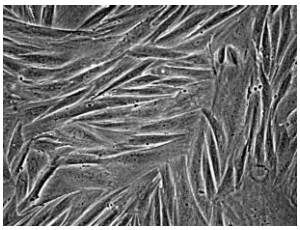
RA#1



Day 106 - 20X Objective

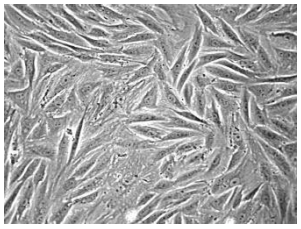


Day 128 - 20X Objective

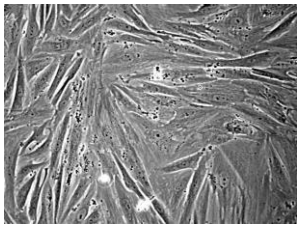


Day 204 - 20X Objective

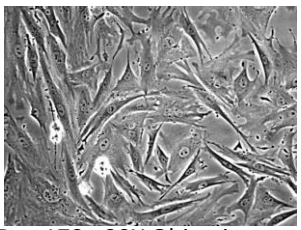
RA#5



Day 48 - 20X Objective

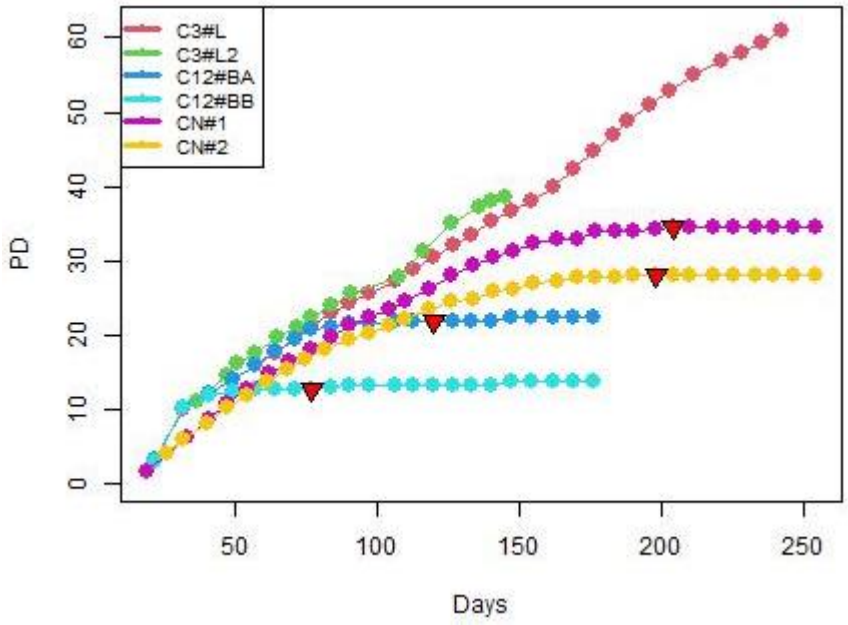


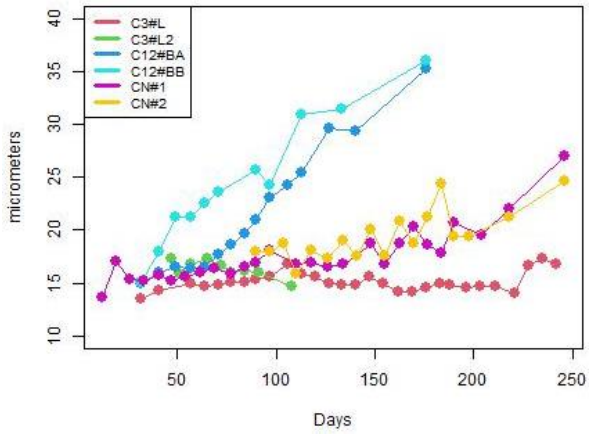
Day 139 - 20X Objective



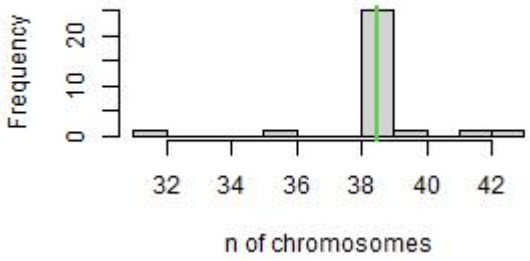
Day 170 - 20X Objective

Felis catus (Domestic cat - Tabby)

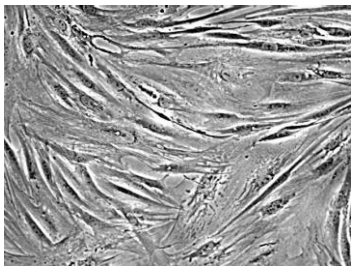




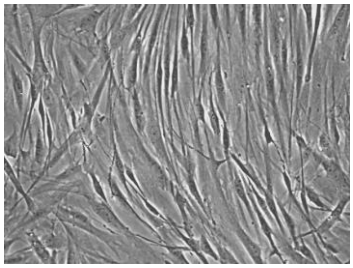
C3#L2 at PD 25.60 (2n=38)



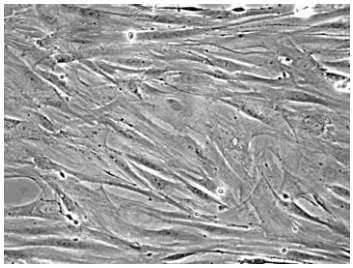
C3#L



Day 147 - 20X Objective

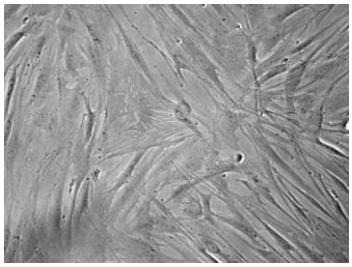


Day 188 - 20X Objective

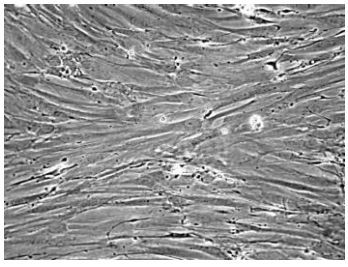


Day 242 - 20X Objective

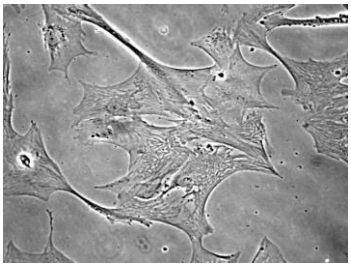
CN#2



Day 40 - 20X Objective

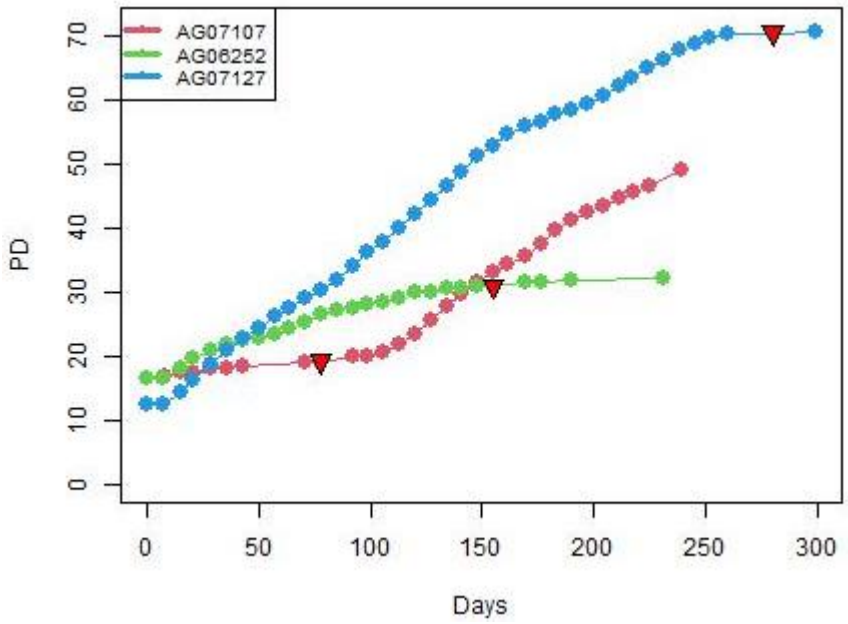


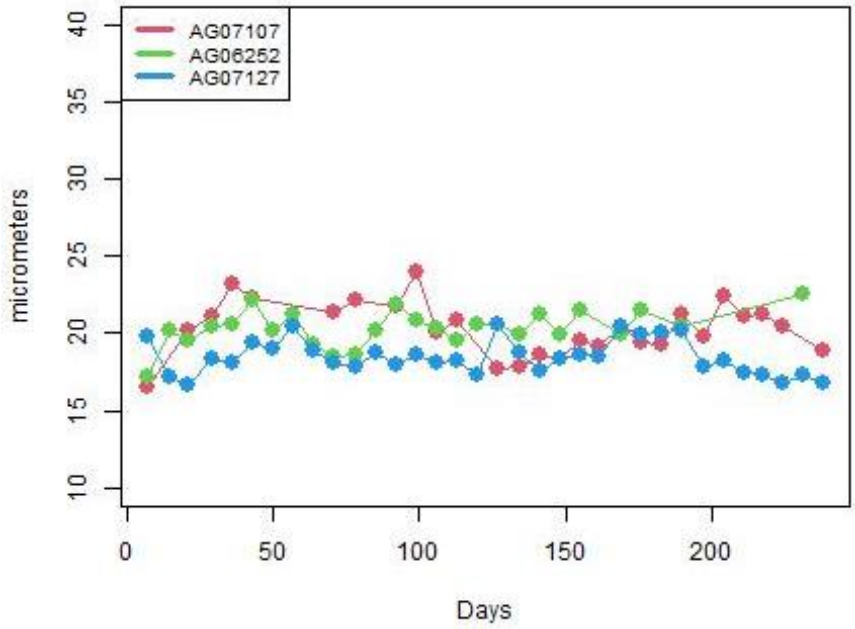
Day 110 - 20X Objective



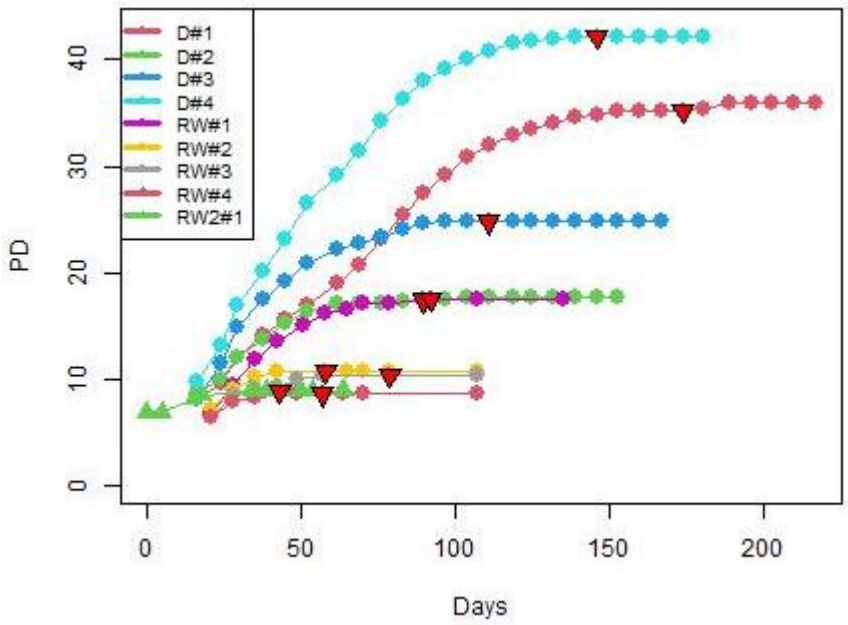
Day 221 - 20X Objective

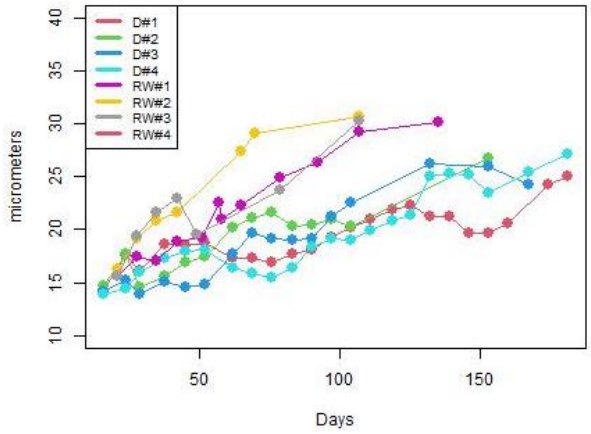
Macaca mulatta (Rhesus monkey)



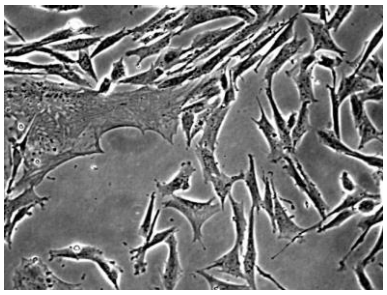


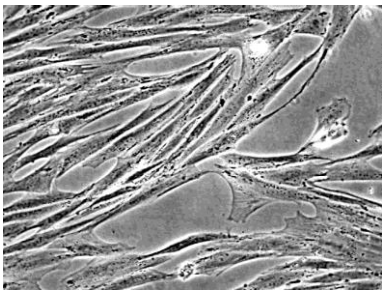
Canis lupus familiaris (Dog)

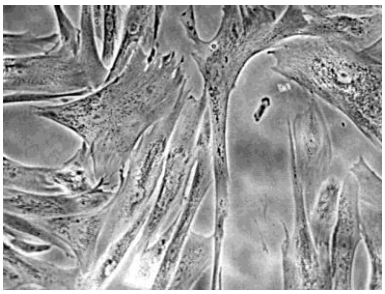




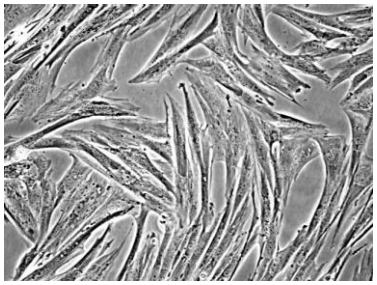
D#1

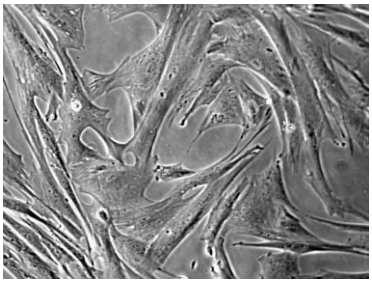

Day 16 - 20X Objective

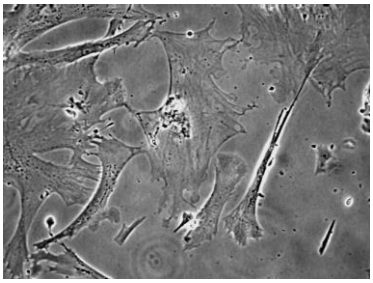

Day 90 - 20X Objective


Day 209 - 20X Objective

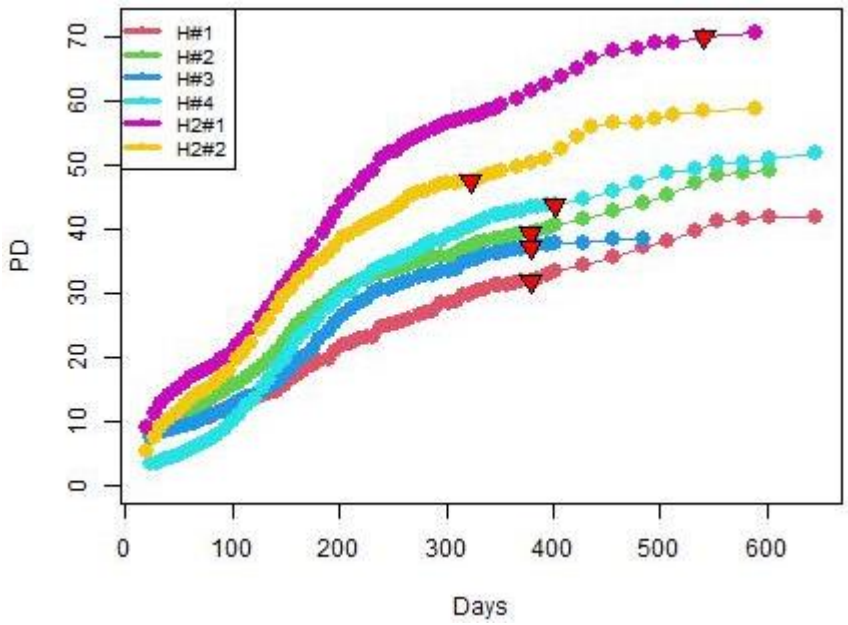
RW#2

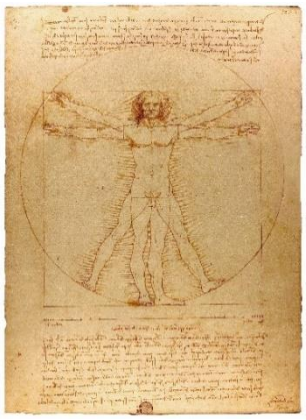

Day 27 - 20X Objective

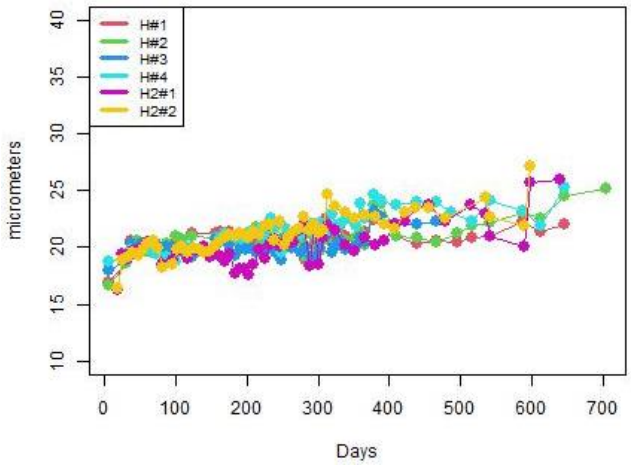

Day 34 - 20X Objective


Day 107 - 20X Objective

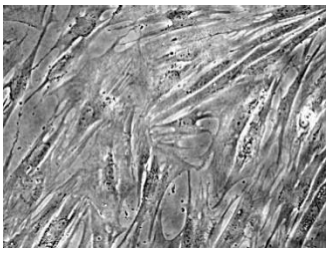
Homo sapiens (Human)



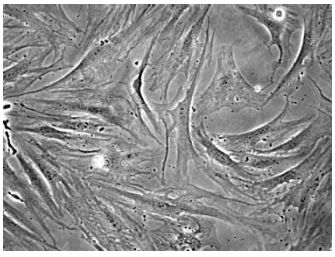




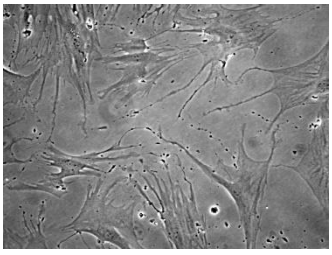
H#1



Day 40 - 20X Objective

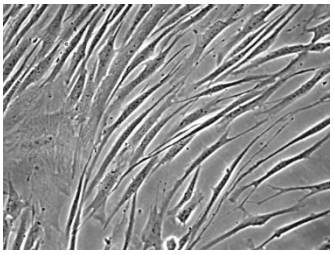


Day 240 - 20X Objective

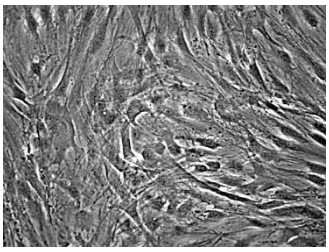


Day 437 - 20X Objective

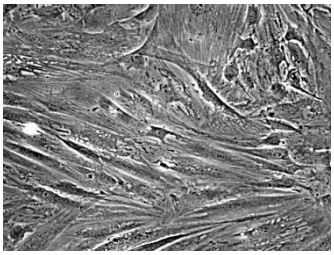
H2#1



Day 20 - 20X Objective

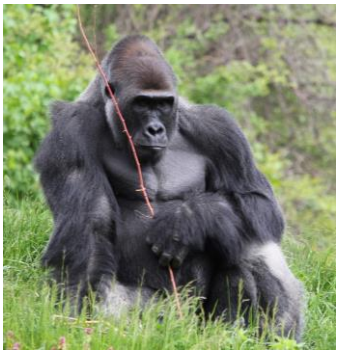


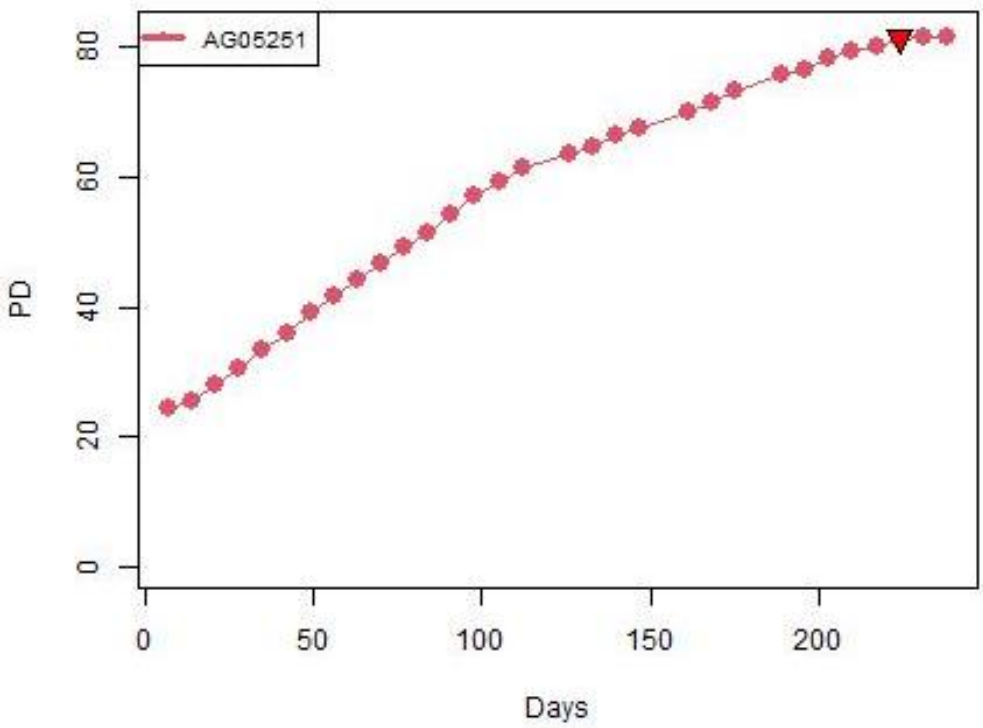
Day 176 - 20X Objective



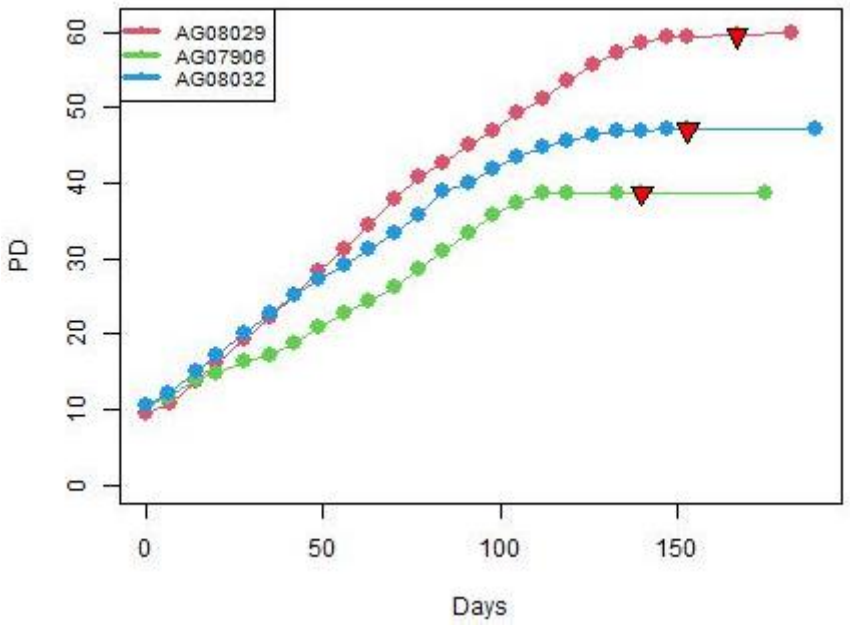
Day 352 - 20X Objective

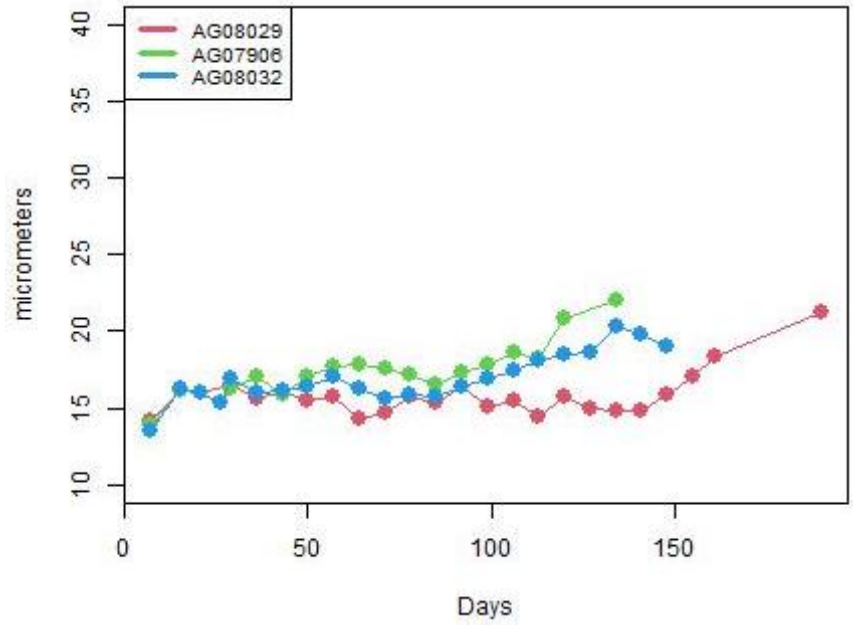
Gorilla gorilla (Low land gorilla)



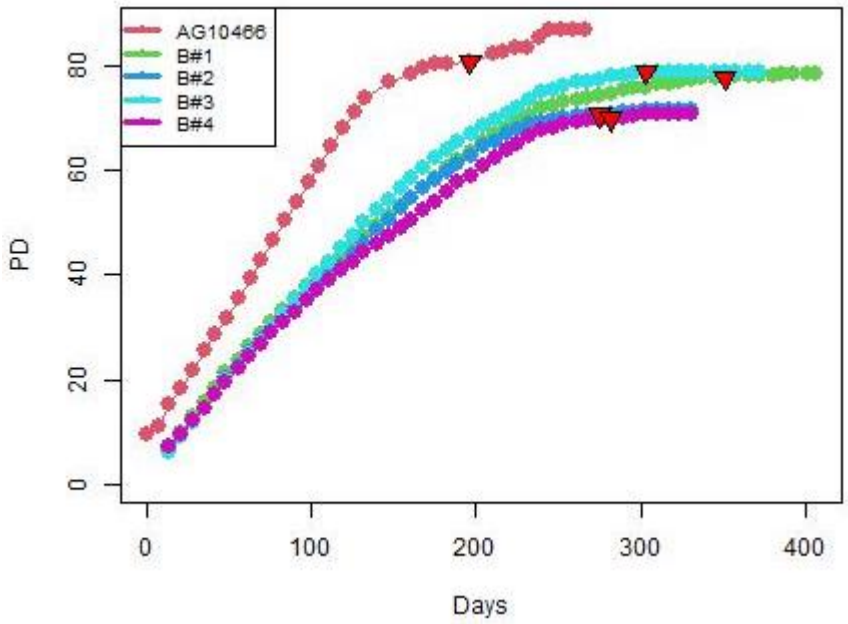


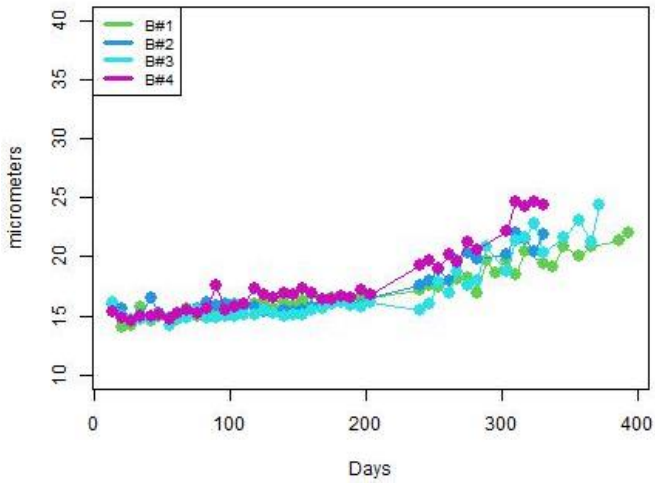
Equus ferus caballus (Horse)



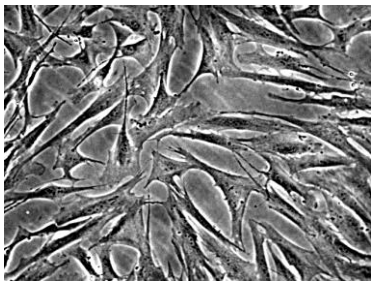


Bos taurus (Cattle - Holstein)

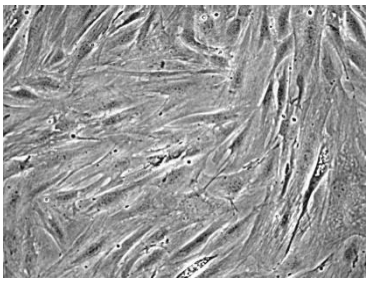




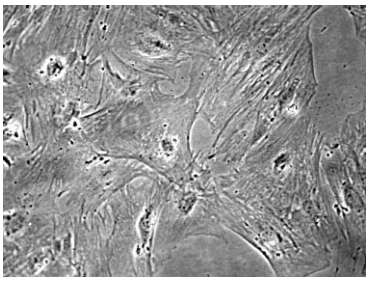
B#1



Day 14 - 20X Objective

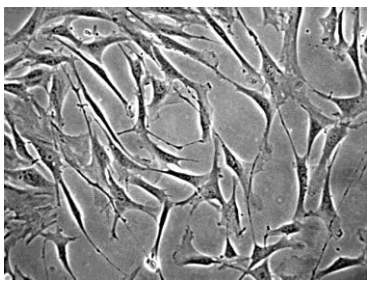


Day 139 - 20X Objective

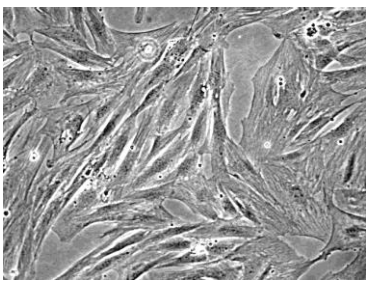


Day 372 - 20X Objective

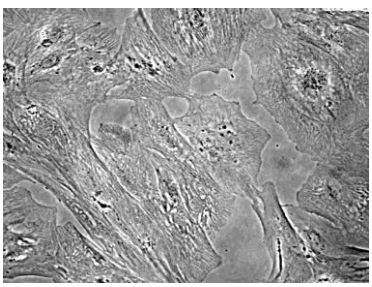
B#4



Day 14 - 20X Objective



Day 139 - 20X Objective



Day 324 - 20X Objective