

Supplementary data

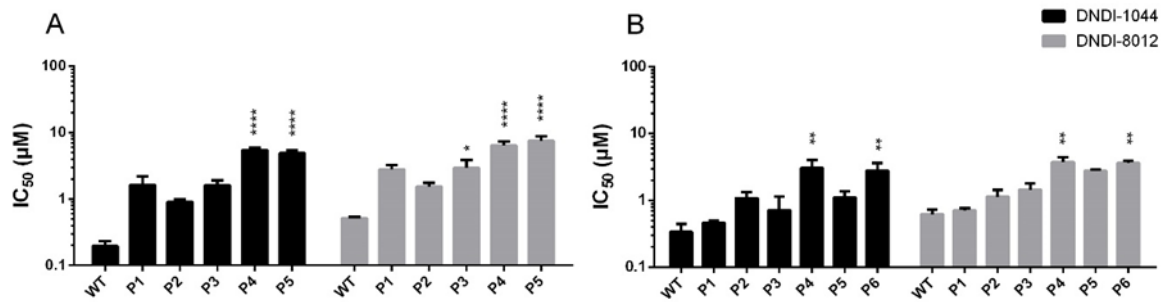


Figure S1: Comparison of the *in vitro* susceptibility in the extracellular promastigote assay of the wild type parent (WT) with selected lines LEM3323Cl₄/1044 (A) and LEM3323Cl₄/8012 (B) at each passage following exposure to the APs DNDI-1044 and DNDI-8012. Results are expressed as the mean IC₅₀ (µM) ± SEM and are based on two independent replicates run in duplicate. (*: p < 0.05, **: p < 0.01, ****: p < 0.0001).

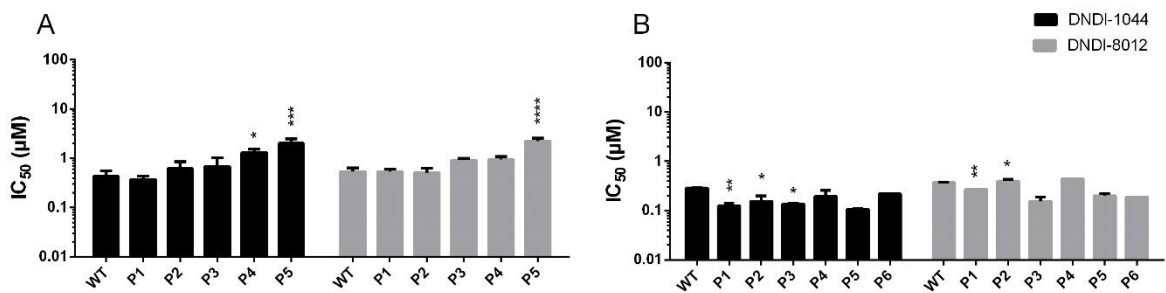


Figure S2: Comparison of the *in vitro* susceptibility in the intracellular amastigote assay of the wild type (WT) parent with selected lines LEM3323Cl₄/1044 (A) and LEM3323Cl₄/8012 (B) at each passage following exposure to the APs DNDI-1044 and DNDI-8012. Results are expressed as the mean IC₅₀ (µM) ± SEM and are based on two independent replicates run in duplicate. (*: p < 0.05, **: p < 0.01, ***: p < 0.001, ****: p < 0.0001).

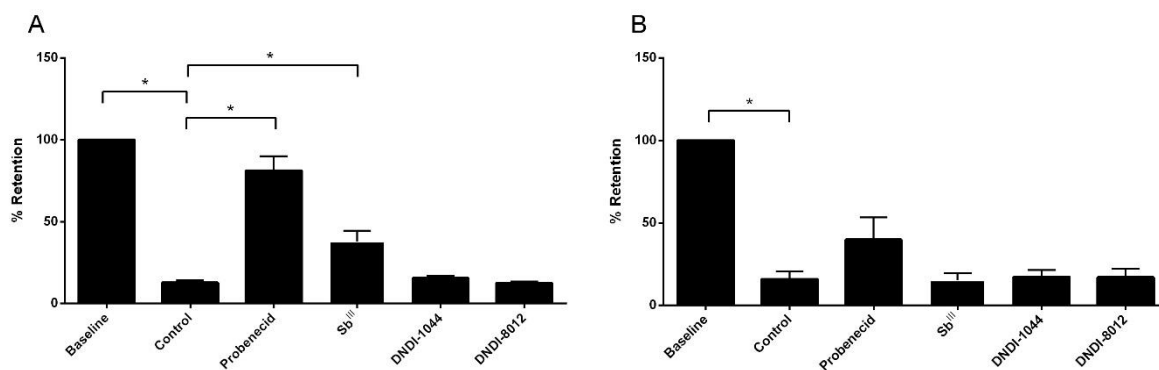


Figure S3: Percentage retention of 5(6)-carboxy-2'-7'-dichlorofluorescein (CDCF) in uninfected (A) and infected (B) macrophages after 2h of efflux with probenecid or the aminopyrazoles. Results are based on two independent repeats run in duplicate and are expressed as the mean of the $IC_{50} \pm$ the standard mean of error (SEM) (* $p < 0.05$).

Table S1. Chemical structure of the aminopyrazole series.

DNDI-1044	
DNDI-8012	
DNDI-5561	

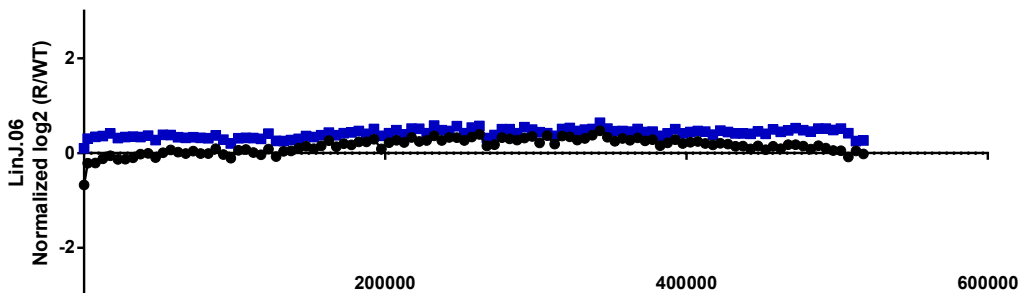
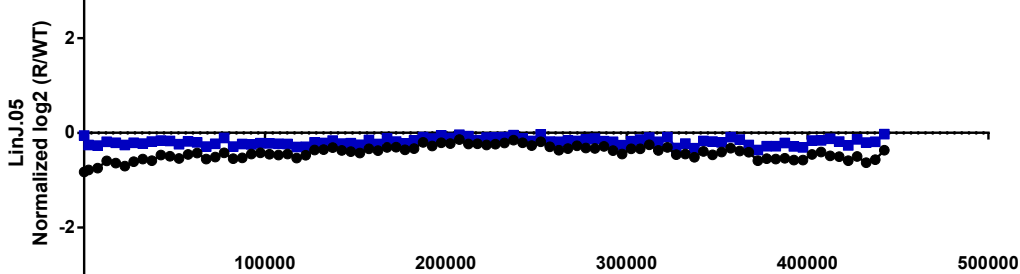
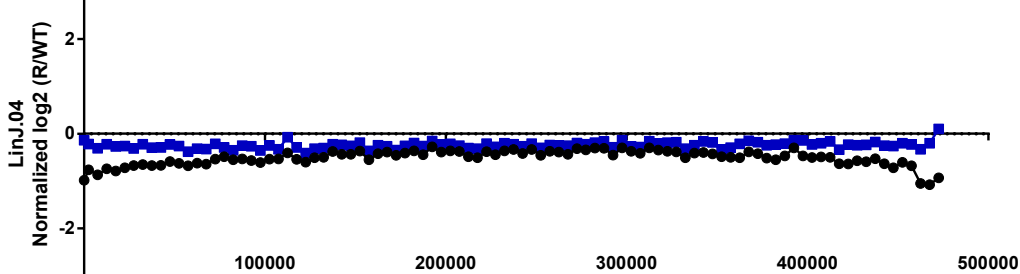
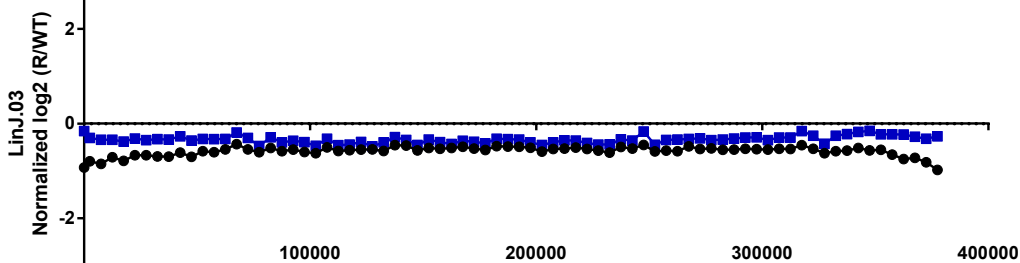
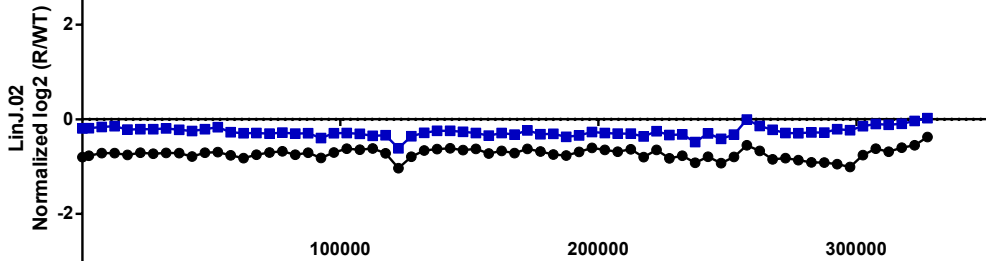
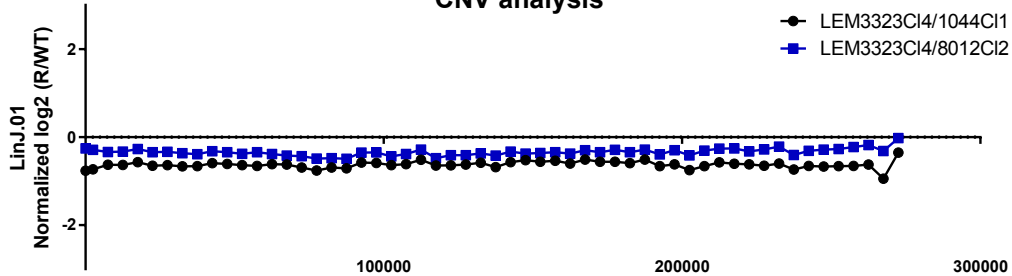
Table S2: Mean macrophage infection rate and infection ratio of both the resistant strains compared to the wild type line at 24hpi. Results are expressed as the mean percentage infected macrophages or infection ratio (#amastigotes/#macrophages) \pm standard error of mean (SEM) and are based on one experiment run in sextuplet.

24hpi	LEM3323Cl ₄	LEM3323Cl ₄ /1044Cl ₁	LEM3323Cl ₄ /8012Cl ₂
% Infected macrophages	87 \pm 3	86 \pm 2	87 \pm 5
Infection ratio	6.2 \pm 0.8	7.7 \pm 0.6	6.1 \pm 0.8

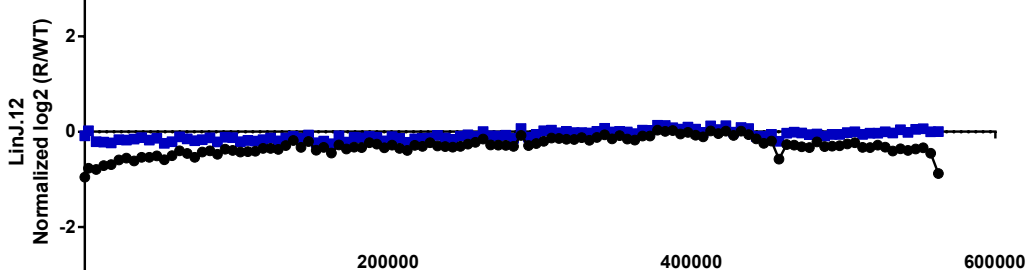
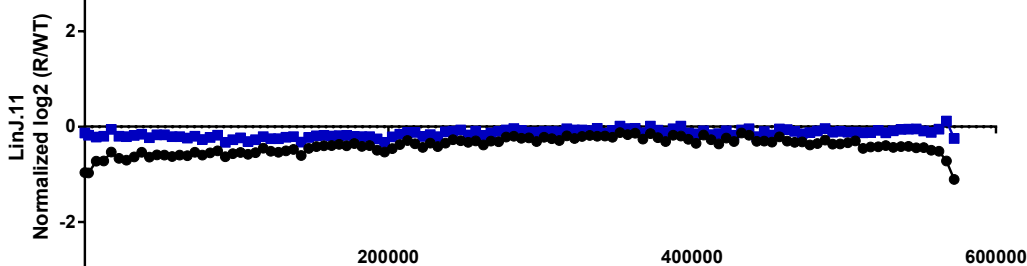
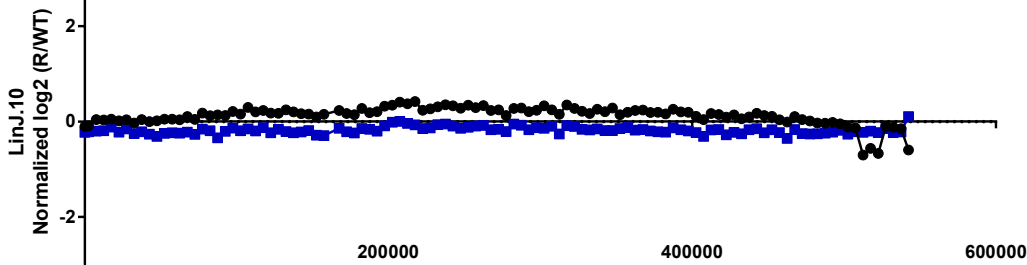
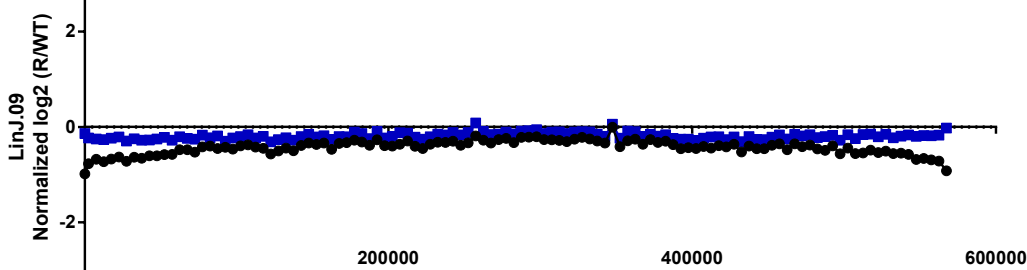
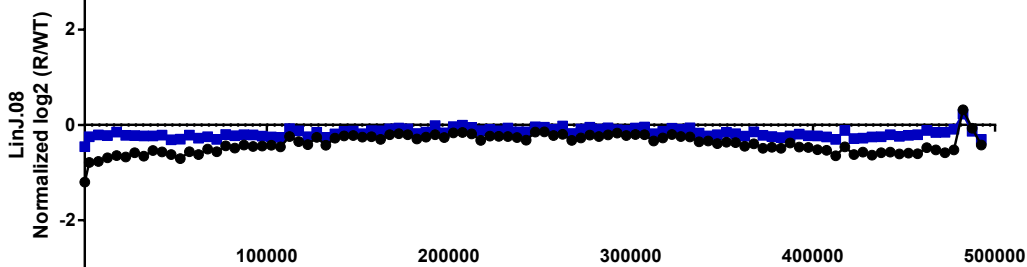
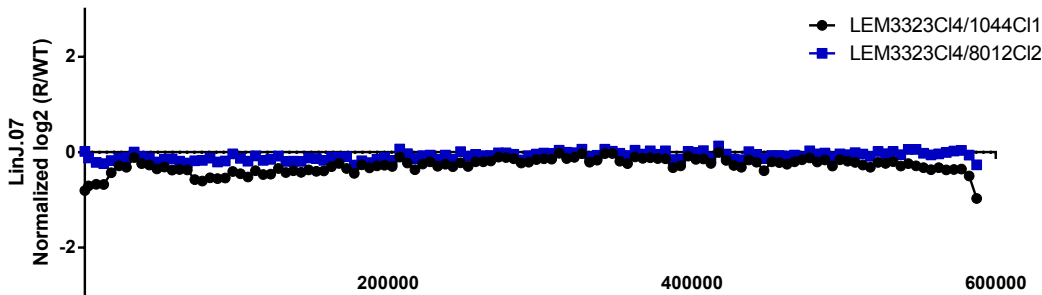
Table S3: *In vitro* activity of the efflux pump inhibitors on intracellular amastigotes and extracellular promastigotes of the *L. infantum* and *L. donovani* laboratory strains. Results are expressed as the mean IC₅₀ (μ M) \pm standard error of mean (SEM) and are based on two independent replicates run in duplicate (*n*=4).

		Verapamil	Cyclosporine A	Probenecid
Intracellular amastigote assay	<i>L. infantum</i>	10 μ M	2 μ M	> 4.5 mM
	<i>L. donovani</i>	10 μ M	4 μ M	> 4.5 mM
	Cytotoxicity	64 μ M	10 μ M	> 4.5 mM
Extracellular promastigote assay	<i>L. infantum</i>	100 μ M	14 μ M	875 μ M
	<i>L. donovani</i>	40 μ M	15 μ M	875 μ M

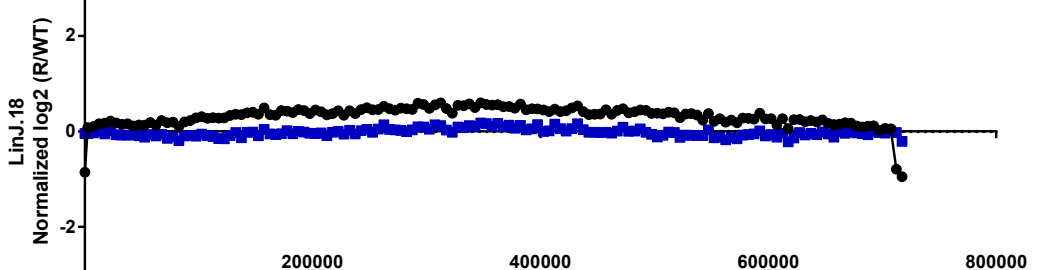
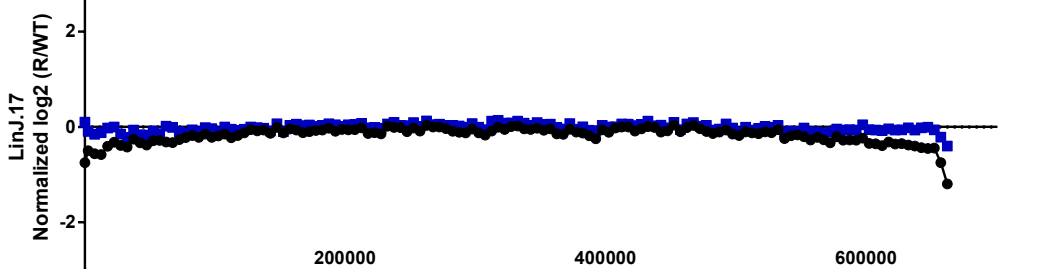
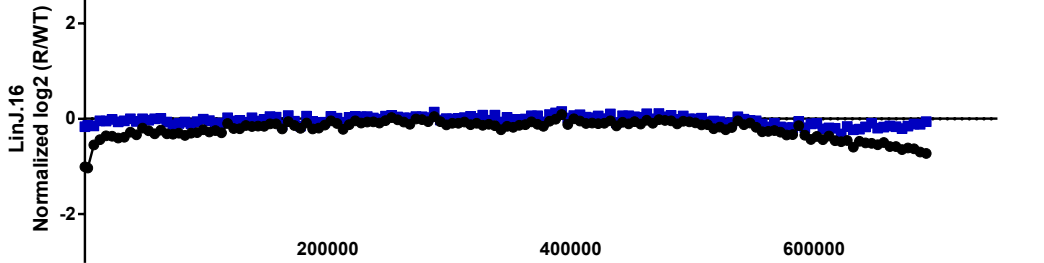
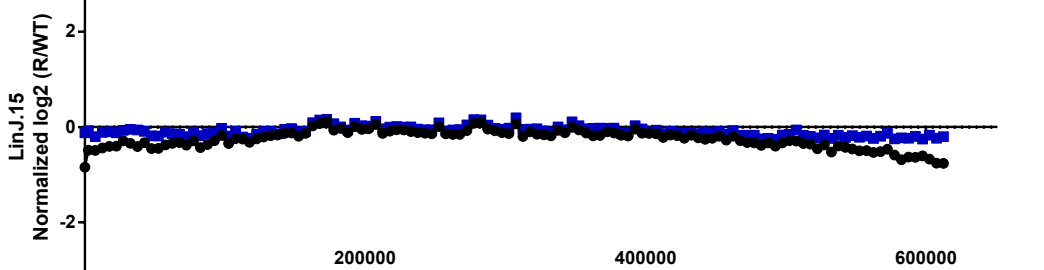
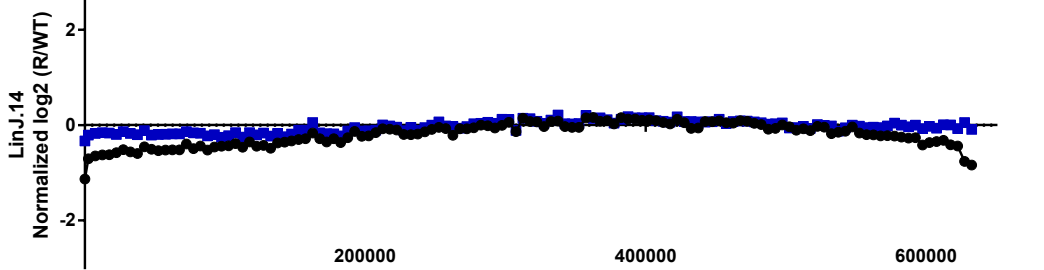
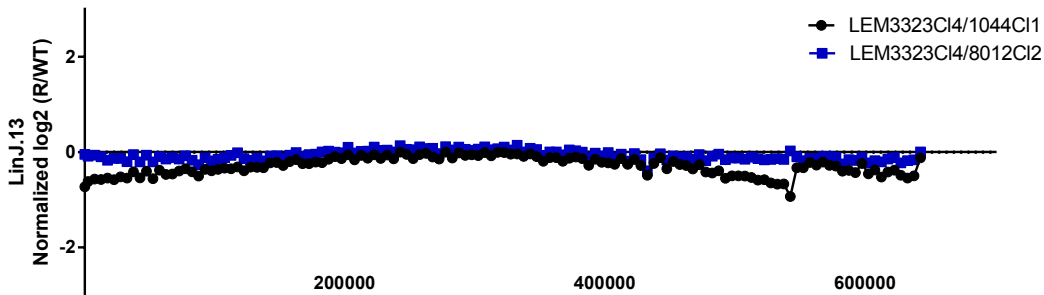
CNV analysis



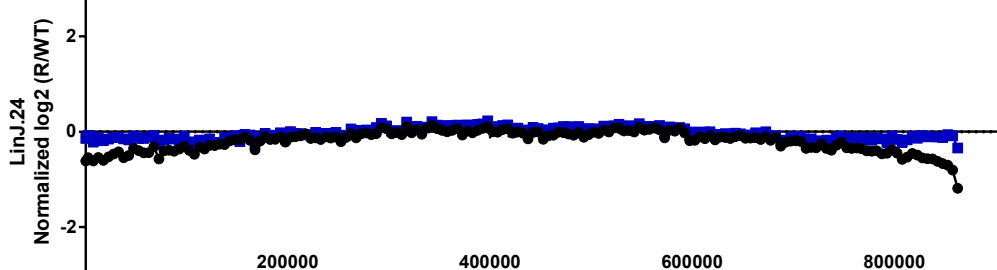
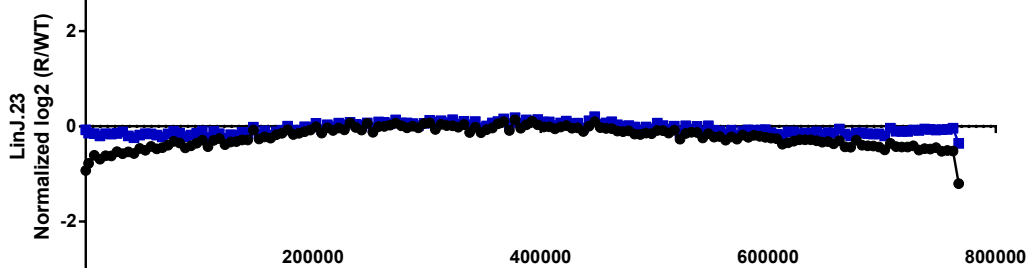
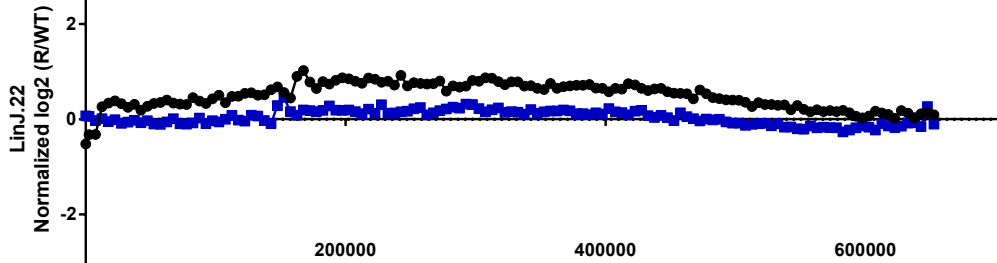
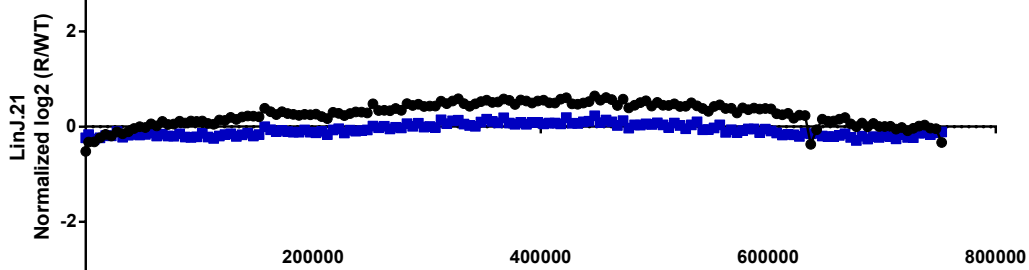
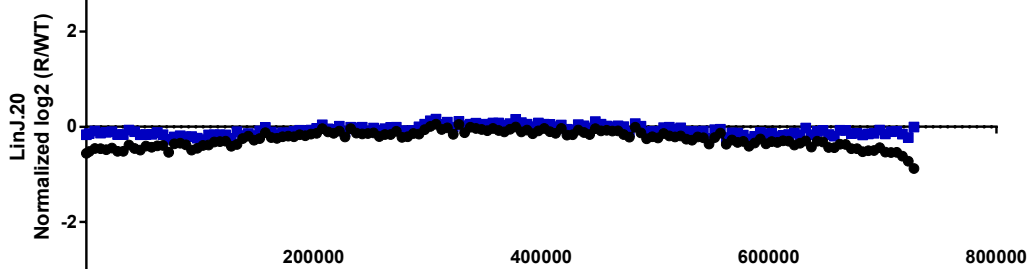
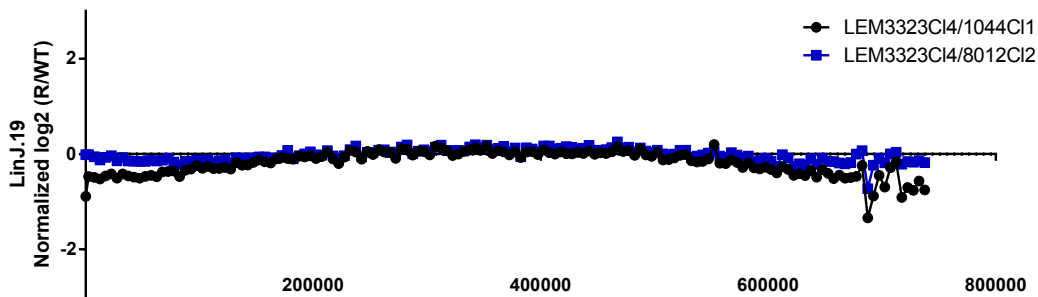
Chromosome position



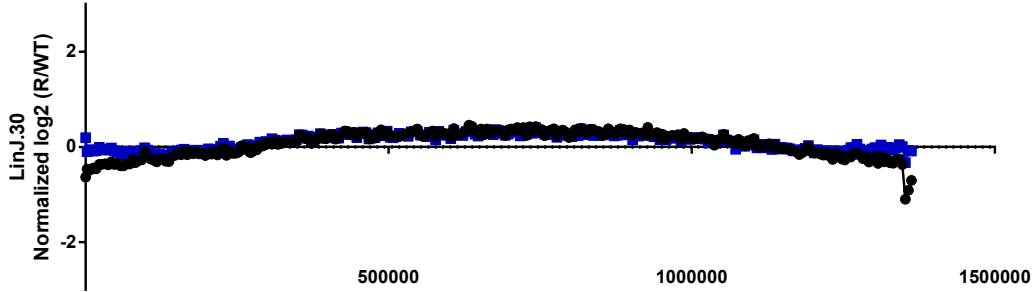
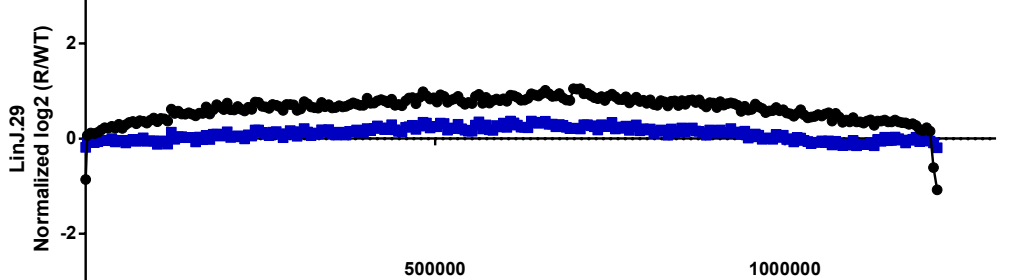
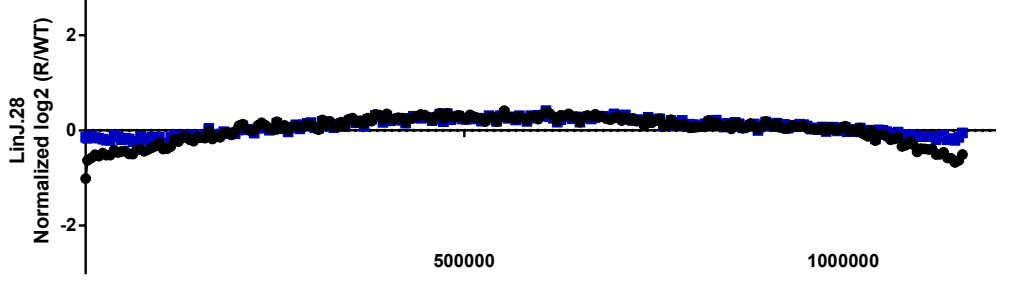
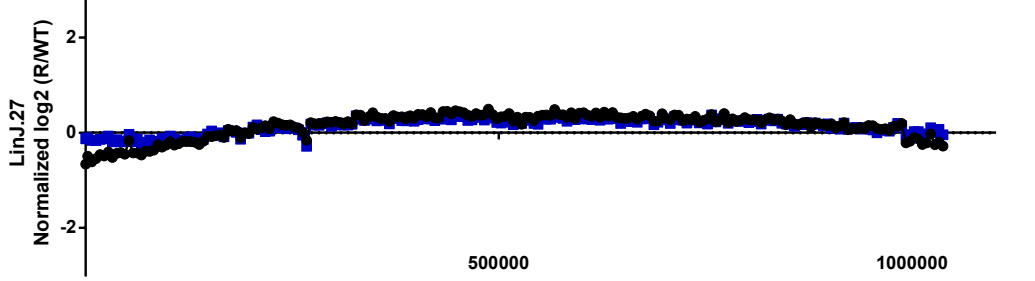
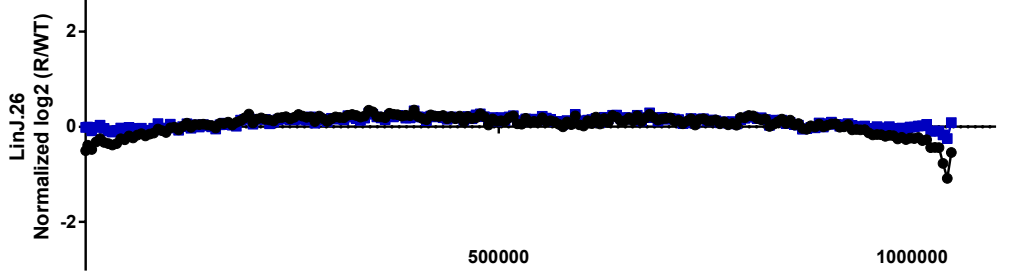
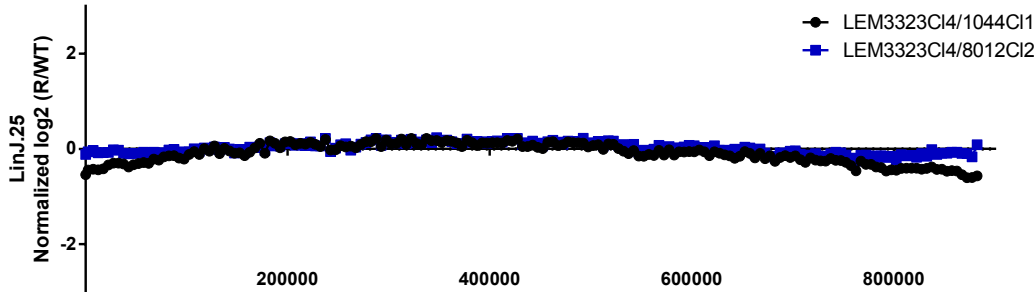
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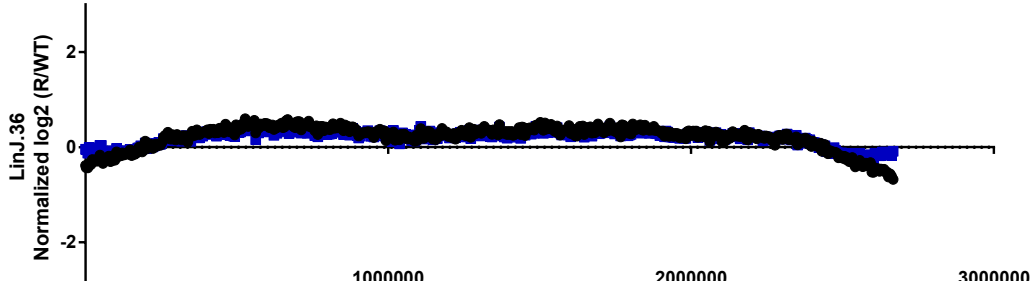
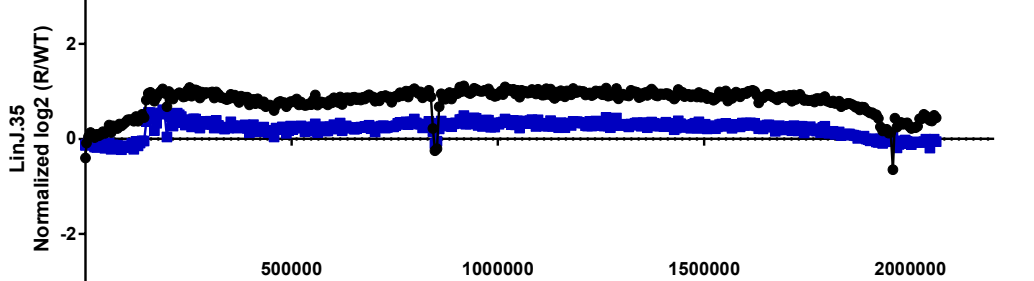
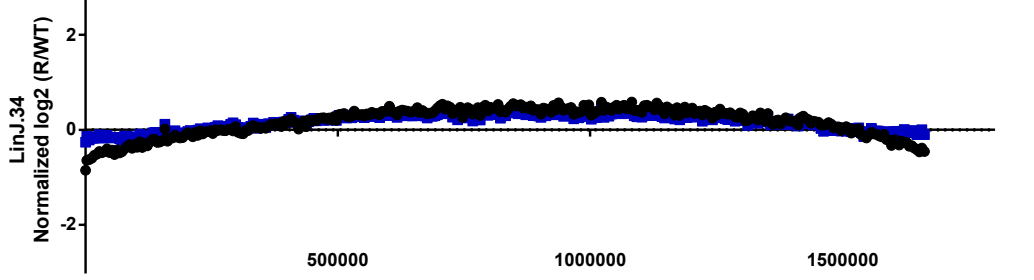
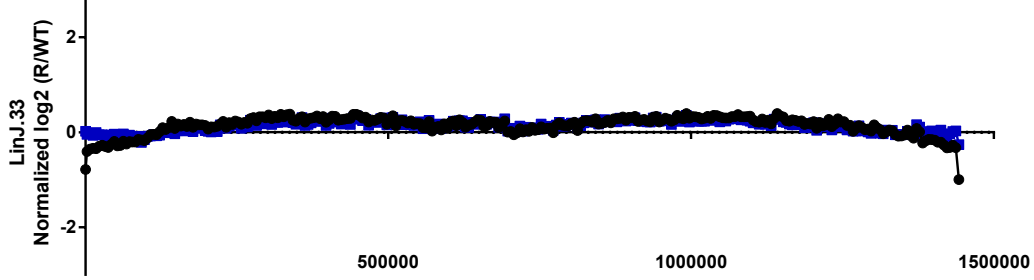
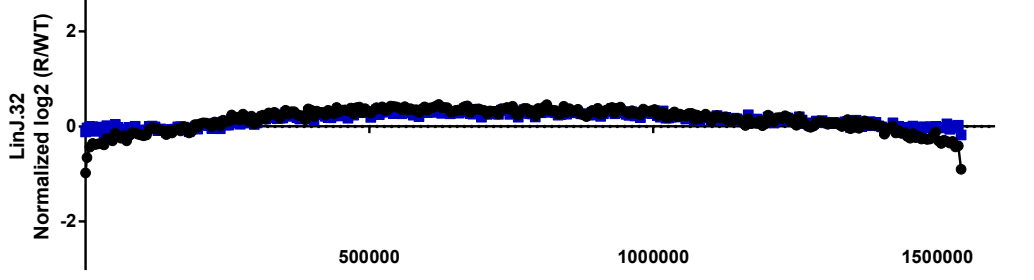
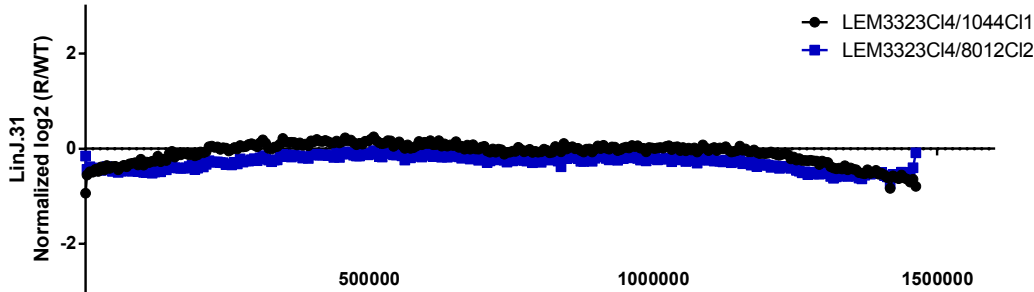
Chromosome position



Chromosome position



Chromosome position



Chromosome position