Figure S3
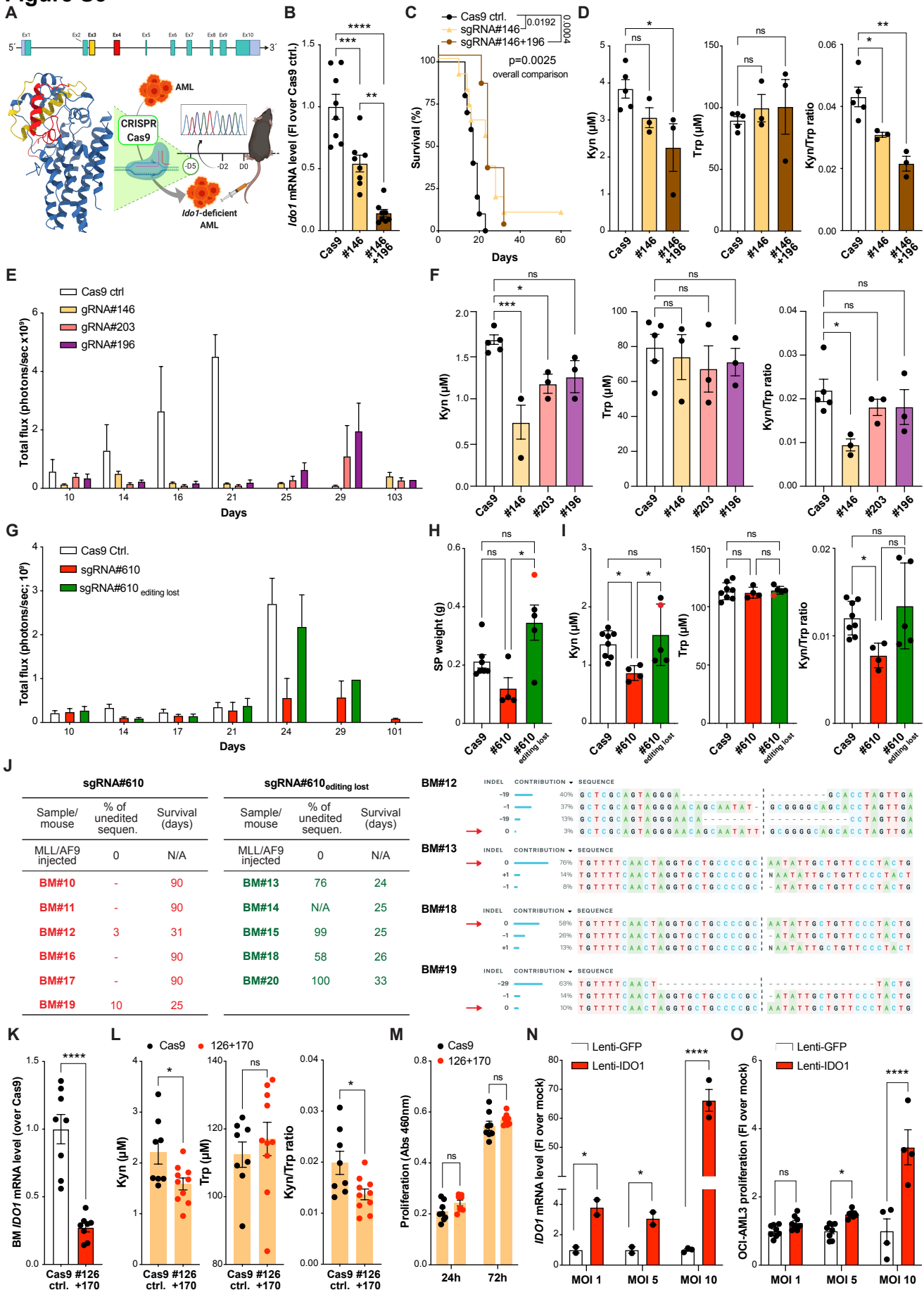
Supplementary Figure S3. CRISPR-Cas9-mediated genetic inactivation of *IDO1* in AML abrogates leukemia progression. Related to Figure 3. (**A**) CRISPR/Cas9-mediated *Ido1* disruption strategy. Left: human IDO1 structure (PDB 6E45) with exons 3 (yellow) and 4 (red) highlighted; right: *Ido1* locus on top and schematic of the experiment below. (**B**) *Ido1* mRNA levels in WEHI-3B-edited cells (n=8). (**C**) Survival of mice injected with WEHI-3B-edited cells nucleofected with Cas9 only (n=10), sgRNA#146 (n=11) or sgRNA#146+196 (n=6); Kaplan-Meier curve with p-value of log rank (Mantel-Cox) test. (**D**) Systemic Kyn, Trp and Kyn/Trp ratio levels of WEHI-3B edited cells, Cas9 control (n=5), sgRNA#146 (n=3) or sgRNA#146+196 (n=3). (**E**) Leukemia burden of exon 3-targeted MLL/AF9 cells (n=3/group). (**F**) Systemic Kyn, Trp and Kyn/Trp ratio levels in exon-3-targeted MLL/AF9 cells (n=3/group). (**G**) Leukemia burden quantification of exon 4-targeted MLL/AF9 cells (n=8-11). Spleen weight (**H**) and systemic Kyn, Trp and Kyn/Trp ratio levels (**I**) in exon 4-targeted MLL/AF9 cells, Cas9 control (n=8), sgRNA#610 (n=4), sgRNA#610 editing lost (n=5); red dot: mouse#19. (**J**) Table summarizing % of un-edited (WT) sequence in the BM at harvest *versus* % survival in mice injected with sgRNA#610 (red, n=6/11) or sgRNA#610$_{\text{editing lost}}$ (when WT sequence in BM at harvest is >60%) (green, n=5/11). Mice that never developed leukemia (#10, #11, #16 and #17) were harvested at day 90; #14 died before harvest. The trace files show the relative contribution of each indel sequence inferred from the indicated BM. Arrow indicates WT sequence. BM *IDO1* mRNA level (**K**) and systemic Kyn/Trp levels (**L**) in NSG mice 3 weeks after OCI-AML3 injection (Cas9 n=8; sgRNA#126+170 n=10); unpaired t-test. (**M**) OCI-AML3 proliferation at 24 and 72h (n=8); two-way ANOVA. (**N**) *IDO1* mRNA level (n=2-3; unpaired-t-test) and proliferation (**O**) at 24h (n=4-8; two-way ANOVA) of OCI-AML3 transduced with lentiviral particles at the indicated MOIs carrying either mock or IDO1-overexpressing vectors. All data are presented as mean ± SEM. Statistical analysis done by one-way ANOVA unless otherwise indicated.