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POSTER ABSTRACTS

503.CLONAL HEMATOPOIESIS, AGING AND INFLAMMATION

In Vivo CRISPR Screening Platform for Studying Myeloid Inflammation and Clonal Dynamics

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Previous experimental approaches using CRISPR to study factors governing hematopoiesis *in vivo* have been hampered by heterogeneity in cellular expansion during hematopoietic reconstitution. To control for such 'cellular jackpotting', we developed an *in vivo* hematopoietic stem and progenitor (HSPC)-based CRISPR screening system with unique molecular identifiers (UMIs) and applied these techniques to study myeloid inflammation and clonal expansion of *Tet2*-mutated HSPCs. Transplantation of Cas9-expressing Lin $^{-}$Sca1 $^{+}$c-Kit $^{+}$ (LSK) cells transduced with high-diversity UMI-labeled guide-RNAs (gRNAs) enabled sufficient gRNA representation at a genome-wide level after lentiviral integration of one gRNA per cell and controlled for 10 log-fold differences in cellular expansion during hematopoietic reconstitution. Simultaneous comparison of barcoded CRISPR screening with non-barcoded screening revealed that barcoding decreases false-positive biological processes related to cell division while improving sensitivity for detecting biological processes related to transcriptional events and cellular differentiation. To study inflammatory mediators, we applied this CRISPR screening tool towards studying the recruitment of CD101 $^{+}$Ly6G $^{+}$CD11b $^{+}$ mature neutrophils during an inflammatory insult, which revealed the majority of genes involved in human congenital neutropenia (Figure A). To demonstrate the reproducibility of this screening system, we performed a targeted transcription factor (TF)-based CRISPR screen, which revealed concordance between the genome-wide screen and the targeted screen. Next, we also applied this screening system to studying dynamics of *Tet2*-mutated clonal hematopoiesis. To study master regulators of clonal expansion in *Tet2* KO, we performed a UMI-based CRISPR screen using chromatic and transcription factors, which revealed *Tet3* and *Bcor*, genes previously identified to cooperate with *Tet2* KO to promote myeloproliferation (Figure B). Validation of additional novel genetic hits is currently ongoing. We hypothesize that this screening platform will be useful for dissecting many aspects of hematopoiesis including inflammatory mediators and clonal dynamics of HSPCs.

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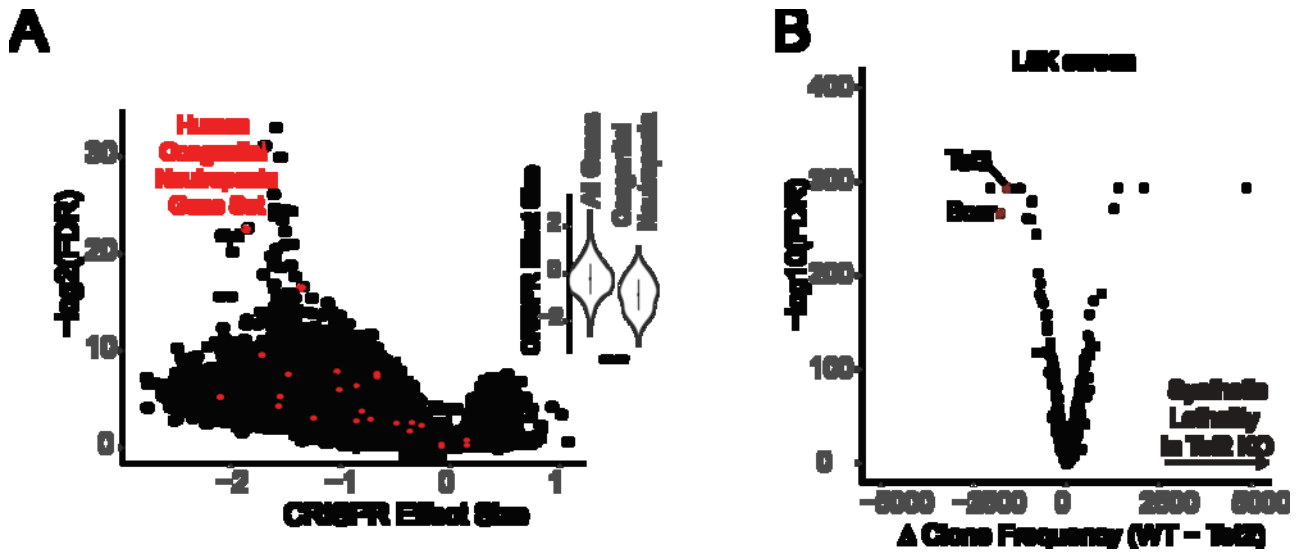

Figure 1

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