Supp table S1

Gene	Protein type (eg TF or cell surface)	Type of Mouse (deleter)	Pre-TP defect	Altered FL hemo	TPation assays performed	Severe in vivo defect	moderate in vivo defect
Adar1	Adenosine deaminase; transcript editing	cKO (Mx1 and Tam)		+	+	+	
AHR	bHLH TF	KO	+		+		
Akt1	PTK	KO			+		
Akt1 and Akt2	PTK	dKO		+	+		+
Akt2	PTK	KO			+		
Als2	GEF for Rab5	KO					
Apc	TS	cKO (Mx1- Cre)	+		+	+	
Arhgap1	GAP	KO	+	+	+	+	
Atg7	autophagy	cKO (Vav-Cre)	+	+	+	+	
Atm	DNA damage checkpoint	KO			+	+	
Bmi1	Polycomb complex	KO			ND		
Bmi1	Polycomb complex	KO			+	+	
c-Myc	TF	cKO (Mx1Cre)	+		+	+	
c-Myc	TF	cKO (Mx1Cre)	+		+	+	

Table S2. Genes that when knocked out exhibit increased proliferation.

Gene	Type of Mouse	Altered Proliferation (+: increase, –: decrease)	Engraftment Ability in *In vivo* Defective		
			Severe	Moderate	Noticeable but Mild
c-Myc	cKO (Mx1-Cre)	+	+		
c-Myc and N-myc	cKO (Mx1-Cre)	+	+		
FOXO3a	KO	+	+		
Lyl1/SCl double	cKO (Scl $^{fl/fl}$, Mx1-Cre)	+	+		
MLL1	cKO (Mx1-Cre)	+	+		
Mds1/Evi1 (ME)	KO (exon1 LacZ KI)	+	+		
Adar1	cKO (Mx1-Cre)	+	+		
Camk4	KO	+	+		
cdc42	cKO (Mx1-Cre)	+	+		
Cxcr4	cKO (Mx1-Cre)	+	+		
Inpp5d (SHIP)	KO	+	+		
Irgm	KO	+	+		
Lkb1	cKO (Mx1-Cre)	+	+		
Potb1	KO	+	+		
Ptpn11	cKO (Mx1-Cre)	+	+		
Trp53	KO	+	**+ (lymphoma at 4 month)**		
E47	KO	+		+	
FoxO1/FoxO3/FoxO4	Triple cKO (Mx1-Cre)	+		+	
Hoxa9	KO	+		+	
STAT5	cKO (Mx1-Cre)	+		+	
Foxp3	KO	+		+	
Hemp	KO	+		+	
Pbx1	cKO (Tie2-cre & Mx1-cre)	+		+	
Cdkn1c p57(kip2)	cKO (Mx1-Cre)	+		+	
Cdkn1c/Cdkn1b (p57/p27)	dKO	+		+	
Ews	KO	+		+	
Fbxw7	cKO (Mx1-Cre)	+		+	
Tsta3 (FX)	KO	+		+	
lig4	Point Mutant	+		+	
Pten	cKO (Mx1-Cre)	+		+	
Gfi1	KO	+		+	
Txnip	KO	+			+
Egr1	KO	+			
Hif1alpha	cKO (Mx1-Cre)	+			
CD81	KO	+			
Cdkn1a (p21)	KO	+			

Table S3. Recommended technical steps to study HSCs in KO mice

1. Immunophenotypical analysis	
Flow cytometric analysis remains the bedrock of all investigations into the hematopoietic and immune system. A good set of antibodies can reveal differences in the proportion of cell types in the blood and BM, and also major differences in stem and progenitor cell numbers. Importantly, the interpretation of data must reflect the level of specificity conferred by the markers chosen. Many studies still claim alterations in HSC numbers when basing their analysis on "KLS" staining (a.k.a. LKS, KSL, c-Kit+ Sca1+ Lineage$^-$), even though only around 10% of KLS cells are stem cells. Additional markers coupled with KLS are mandatory to use the term "HSC". The most useful combinations include KLS-CD34$^-$Flk2$^-$ (Adolfsson et al., 2001), or the side population coupled with KLS (SP^{KLS}) (Mayle et al., 2012), or KLS-CD150$^+$CD34$^-$ (Wilson et al., 2008). Each of these strategies can be used to identify HSCs to a level of high purity; individually they converge on the same highly overlapping population (Mayle et al., 2012). Rigorous analysis will mark between 0.001% and 0.03% of whole BM cells with the phenotypic characteristics of HSCs, depending on the chosen markers and staining and analysis conditions. Biological replicates and statistical analysis will support purported HSC number differences between WT and KO genotypes. These analyses can also be coupled with short-term progenitor markers (MPP and ST-HSC). However, it is important to note that the immunophenotypical characterization can be misleading in some circumstances. Expression of some of these markers changes dramatically under conditions such as interferon exposure (e.g. Sca1 increases on non-stem cells) or proliferation (c-kit is down regulated on HSCs). Ultimately, changes in HSC numbers must be determined with functional biological assays.	
2. Transplantation analysis	
BM Transplantation Assays – HSC function and frequency	HSCs are functionally defined as cells that can differentiate (give rise to all major types of blood progeny) and to self-renew (generate more stem cells). Thus, a rigorous functional assay is necessary to determine whether HSCs can regenerate the hematopoietic system in an ablated host. The **competitive BM transplantation assay**, in which the tester BM cells (wild type or mutant) are co-transplanted with a set amount of distinguishable whole BM cells, is the most quantitative. Competitor BM not only can rescue the hosts in the context of severe defects of the mutant, but also provides a standard against which different levels of engraftment activity can be measured. The proportion of peripheral blood contributed from the mutant versus competitor BM can be assayed over time, with 4 months being the standard for considering that the blood progeny are derived from transplanted HSCs rather than progenitors. The **limiting dilution assay** will allow the frequency of functional HSCs in whole BM to be determined, helpful in distinguishing increased activity of the same number of HSCs from BM containing more HSCs (Purton and Scadden, 2007). The CD45 allelic system greatly facilitates these assays, and the system works best if the test mice are backcrossed extensively onto the C57Bl/6 background.
Competitive HSC transplantation assays	While we recommend whole BM (WBM) transplantation as the simplest first experiment, competitive transplantation of **purified HSCs** reduces confounding factors introduced by co-transplantation of progenitors and differentiated cells that can influence the outcome. The use of HSCs versus WBM for the transplantation can impact the outcome to a surprising degree (with HSC transplantation often exacerbating a phenotype). For example in one study of a β-catenin (*ctnnb1*) KO, WBM cells from either WT or *ctnnb1*$^{-/-}$ mice were competitively transplanted; no difference in the percentage of engraftment was observed (Cobas et al., 2004).

	However, in a separate study, competitive transplantation of purified HSCs resulted in a drastic reduction in the engraftment of KO HSCs (Zhao et al., 2007). One caveat should be made for HSC transplantation in cases where the immunophenotype of HSCs is altered by the KO; an apparent functional defect may simply result from changed expression of surface marker resulting in poor purification rather than an actual change in HSC number. Thus, WBM and HSC transplantation assays are complementary. Over the past several years there has been increasing interest in the heterogeneity of the HSC compartment (Benz et al., 2012; Muller-Sieburg et al., 2012). While it is not yet part of the standard work-up to investigate alterations in HSC function, we expect increasing attention to mutations that alter preferences for lineage differentiation. Some tests that could indicate an impact on HSC sub-types would be the proportion of phenotypically-defined HSC sub-types present (assayed by Hoechst and/or CD150 staining), but they are primarily defined by differentiation behavior after transplantation of limiting cell numbers.
Serial Transplantation assays – the Self-Renewal Test	The above assays primarily test the ability of the HSCs to generate differentiated progeny. Their self-renewal capacity is determined by **serial transplantation assays** in which engrafted, donor-derived BM cells are subjected to at least one more round of transplantation. Serial transplantation can be done by transplanting whole BM from the primary recipient (Cheng et al., 2000; Dykstra et al., 2007) or purified HSCs (Lin et al., 2011), both of which test the function of previously challenged HSCs to regenerate the hematopoietic system in new hosts. Serial transplantation assays are ideal in competitive settings so that the comparison between wild type and genetically altered HSCs can be quantified in every round of transplantation.
Reciprocal transplantation – the Environmental Influence	Finally, nearly all of the genes discussed in this review were examined purely for cell intrinsic effects on HSC function. The manner in which some of these mutants affect HSC function via extrinsic effects offers an important research opportunity. To test this, WT cells need to be transplanted into KO BM. The result can be highly revealing, but sufficient numbers of KO mice are not always available, so it is not likely to be part of a standard workup unless an extrinsic effect is suspected.
3. Proliferation analysis	
Whenever the cell cycle regulation of the HSC is disrupted, a defect in normal HSC function is likely to arise, compromising their long-term maintenance. Examining how the loss of any given gene alters HSC cell cycle will lead to a better understanding of how the interplay between genetic networks regulates this vital process in stem cells. Thus, we recommend that future studies include cell cycle assessments of KO HSCs by an established protocol (such as Ki-67 staining) capable of analyzing the ratio between quiescent and proliferative HSCs. Moreover, it is essential to characterize the proliferation status of the most homogeneous HSC population (for example, SP^{KSL}) rather than the progenitor compartment (for example, KLS cells) as the progenitor cells (a majority of the KLS population) are already highly proliferative.	