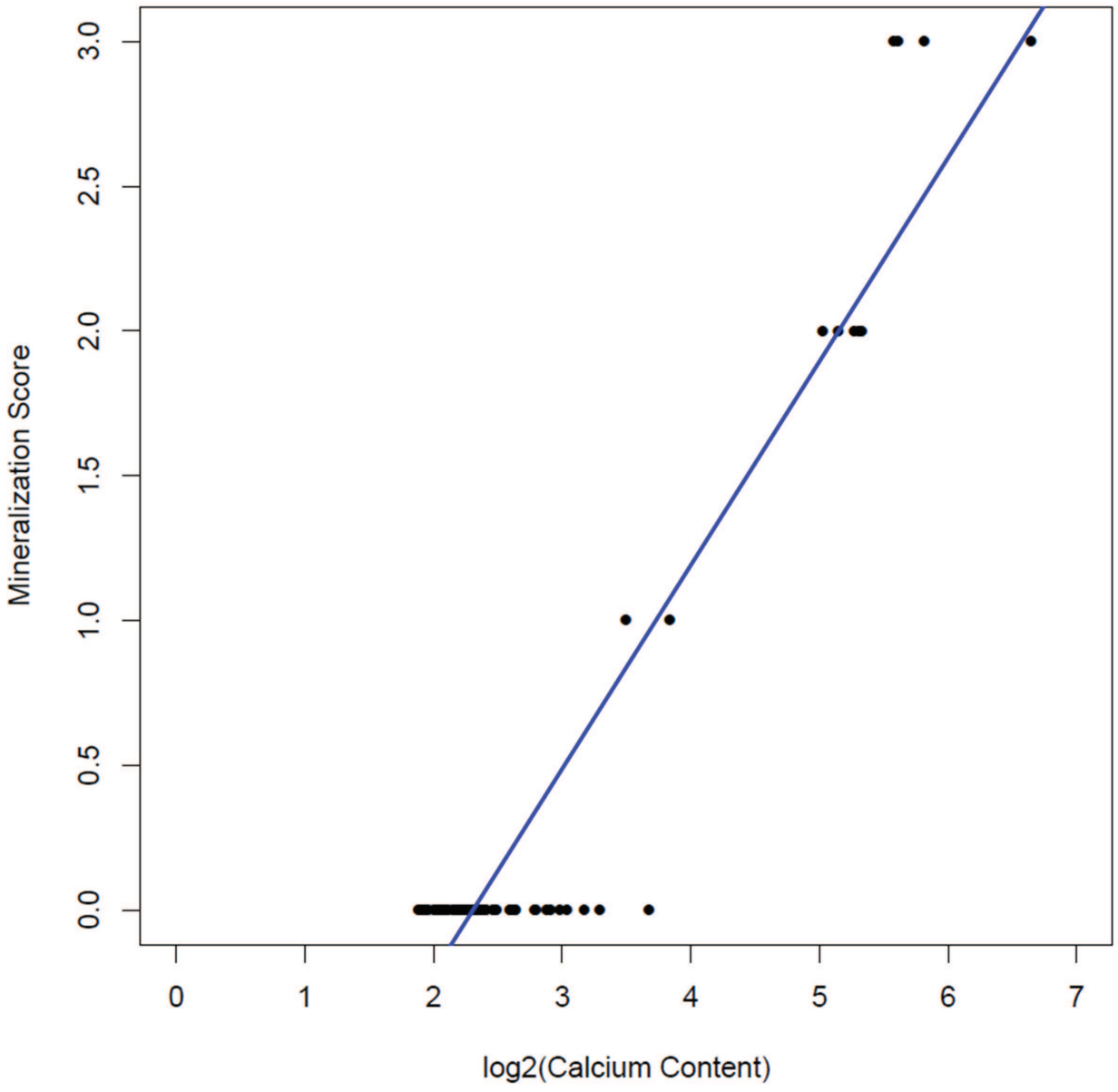
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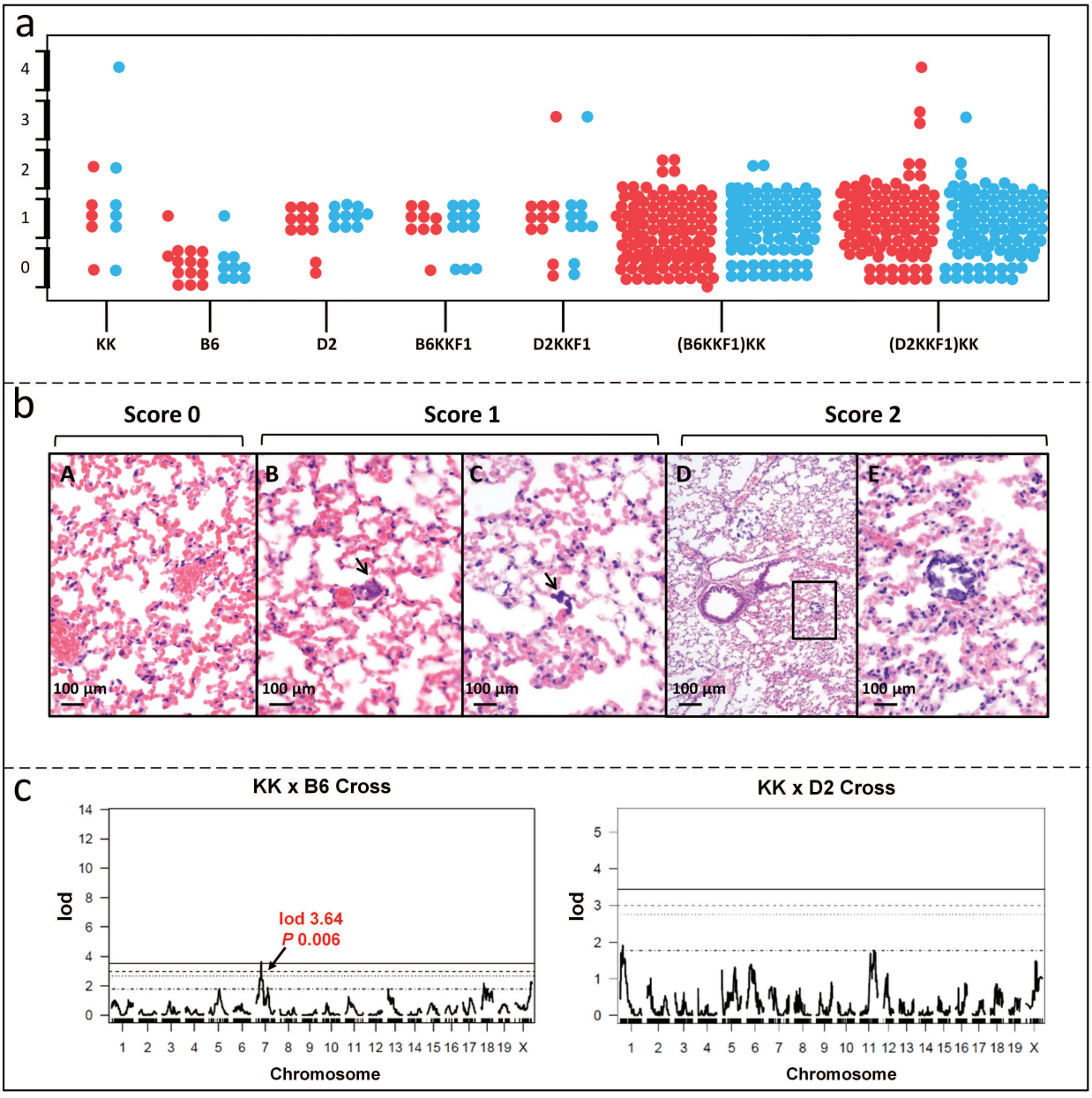
2.0
2.5
3.0
0
1
2
3
4
5
6
7
log2(Calcium Content)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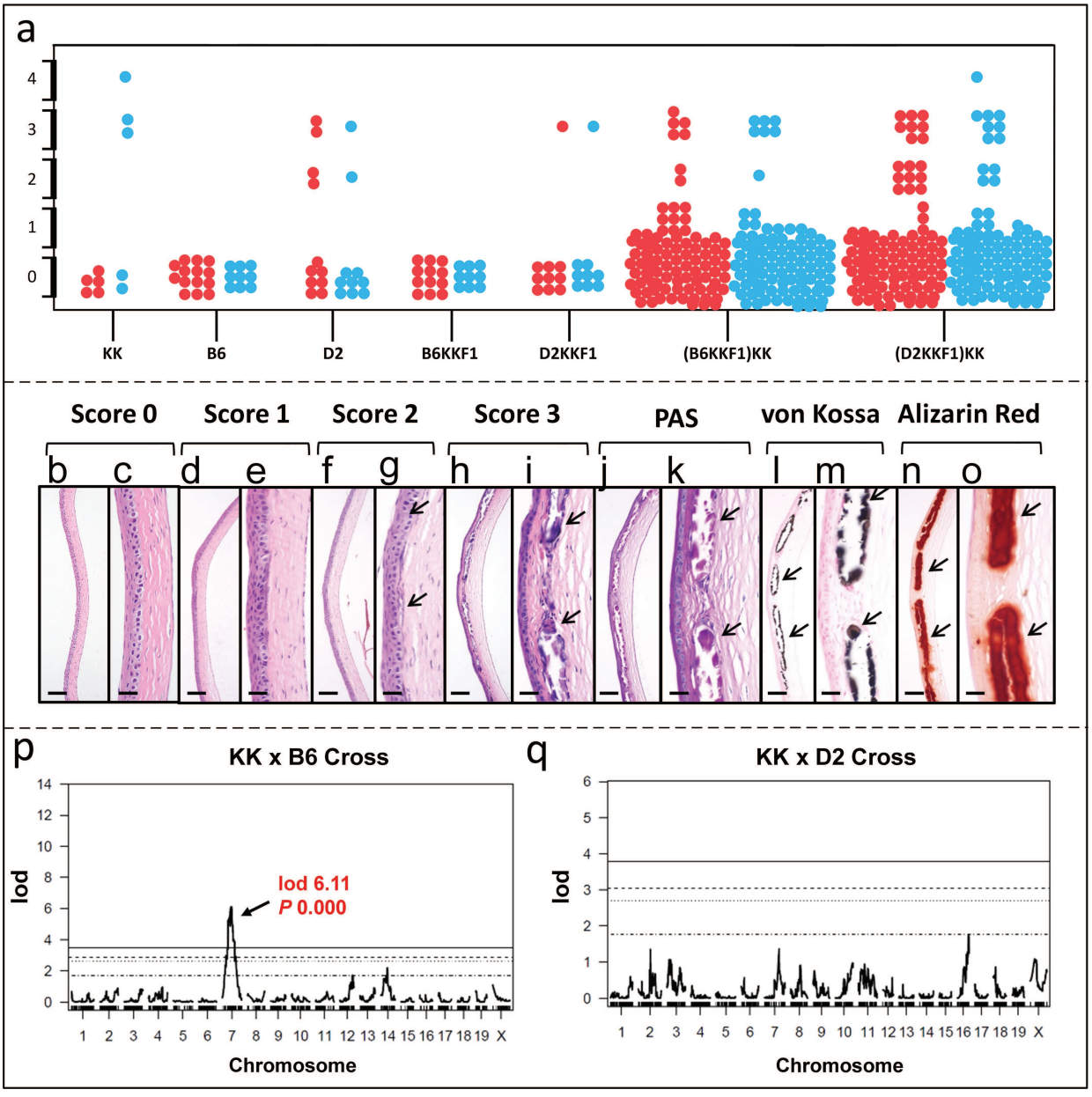
4
3
2
1
0
KK
B6
D2
B6KKF1
D2KKF1
(B6KKF1)KK
(D2KKF1)KK
b
Score 0
Score 1
Score 2
A
B
C
D
E
100 µm
100 µm
100 µm
100 µm
100 µm
c
KK x B6 Cross
lod
lod 3.64
P 0.006
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X
Chromosome
KK x D2 Cross
lod
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X
Chromosome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2
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KK
B6
D2
B6KKF1
D2KKF1
(B6KKF1)KK
(D2KKF1)KK
Score 0
Score 1
Score 2
Score 3
PAS
von Kossa
Alizarin Red
b c d e f g h i j k l m n o
p
KK x B6 Cross
lod
lod 6.11
P 0.000
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X
Chromosome
q
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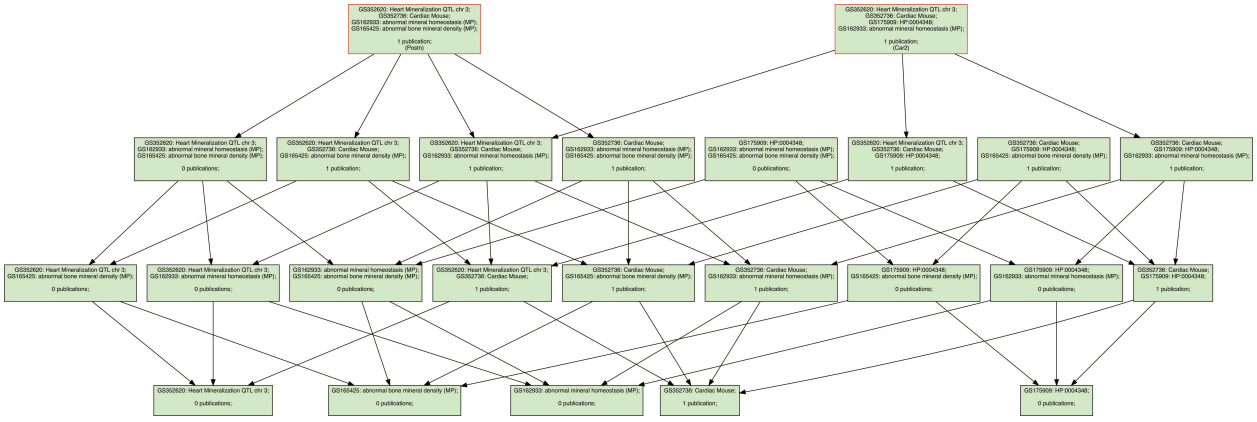
Chromosome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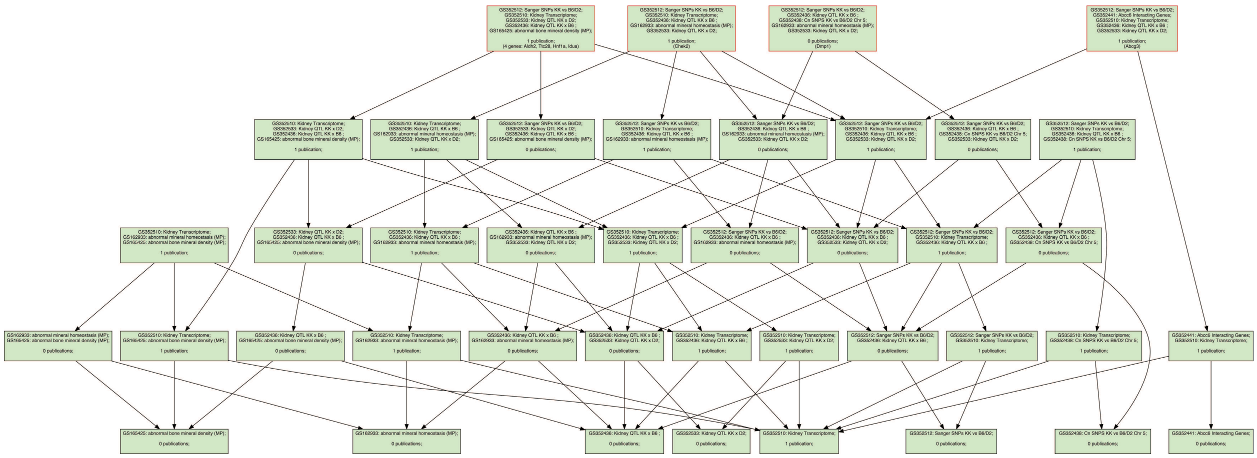
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Table S1. A list of 22 genes, which when mutated in humans and/or mice, result in ectopic mineralization phenotypes. Some of these arose or were made in mouse inbred (congenic) strain backgrounds prone to these conditions, such as C3 or 129 substrains, which carry a hypomorphic allele of *Abcc6* and therefore had lesions that could interfere with interpretation of the phenotype for the gene in question (Berndt *et al.*, 2013; Li *et al.*, 2014b).

Gene Symbol	Gene Name	Human Disease‡	Sites of Ectopic Mineralization		
			Human	Mouse	Ref
Abcc6	ATP-binding cassette, family C, member 6	PXE	skin, eye, kidney, heart, aorta	skin, muzzle skin, eye, kidney, heart, aorta	(Klement *et al.*, 2005; Uitto *et al.*, 2017)
Adipoq	Adiponectin, C1Q and collagen domain containing	UNK	UNK	aorta	(Ma *et al.*, 2002; Streeper *et al.*, 2006)
Ahsg	Alpha-2-HS-glycoprotein	UNK	UNK	skin, heart, kidney, muscle, lung, tongue	(Jahnen-Dechent *et al.*, 1997),(Merx *et al.*, 2005),(Schafer *et al.*, 2003)
Ank	Progressive ankylosis	CMDD	cartilage	cartilage	(Pendleton *et al.*, 2002),(Sweet and Green, 1981),(Villa-Bellosta *et al.*, 2011),(Ho *et al.*, 2000)
Apoe	Apolipoprotein E	Atherosclerosis	aorta	aorta	(Sullivan *et al.*, 1998),(Matsushima *et al.*, 1999)
Atf4	Activating transcription factor 4	Atherosclerosis	aorta	UNK	(Masuda *et al.*, 2016)
Car2	Carbonic anhydrase II	CA II deficiency syndrome	brain	arteries in multiple organs	(Sly *et al.*, 1983),(Spicer *et al.*, 1989)
Casr	Calcium-sensing receptor	HHC1	no lesions	muzzle skin, heart, aorta, kidney, muscle, tongue, testis, ovary, colon	(Hough *et al.*, 2004)
Enpp1	Ectonucleotide pyrophosphatase/phosphodiesterase 1	GACI	arteries	skin, muzzle skin, eye, kidney, heart, aorta, cartilage, ligament, tendon	(Uitto *et al.*, 2017),(Li *et al.*, 2014a),(Li *et al.*, 2013)
Fam20a	Family with sequence similarity 20, member A	UNK	UNK	eye, kidney, heart, lung, muscle, testis	(Vogel *et al.*, 2012)
Fgf23	Fibroblast growth factor 23	HFTC	skin	heart, kidney	(Chefetz *et al.*, 2005),(Shimada *et al.*, 2004)
Galnt3	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3	HFTC	skin	no lesions	(Topaz *et al.*, 2006; Ichikawa *et al.*, 2007)
Ggcx	Gamma-glutamyl carboxylase	PXE/VKCFD1	skin	no lesions	(Li *et al.*, 2009),(Vanakker *et al.*, 2007),(Shiba *et al.*, 2014),(Azuma *et al.*, 2014),(Zhu *et al.*, 2007)
Kl	Klotho	HFTC	skin	skin, aorta, kidney, heart, lung, testis	(Kuro-o *et al.*, 1997),(Ichikawa *et al.*, 2007),(Hu *et al.*, 2011)
Mgp	Matrix Gla protein	KTLS	aorta	aorta, cartilage	(Munroe *et al.*, 1999),(Luo *et al.*, 1997)
Nt5e	5' nucleotidase, ecto	ACDC	arteries, cartilage, ligament, tendon	cartilage, ligament, tendon	(Eltzschig and Robson, 2011),(Li *et al.*, 2014b)

Samd9	Sterile alpha motif domain containing 9	NFTC	skin	no mouse equivalent	(Chefetz *et al.*, 2008),(Topaz *et al.*, 2006),(Sprecher, 2010)
Slc20a2	Solute carrier family 20, member 2	IBGC1	brain	brain	(Jensen *et al.*, 2013), (Jensen *et al.*, 2016)
Slc29a1	Solute carrier family 29 (nucleotide transporters), member 1	UNK	UNK	spinal tissues	(Warraich *et al.*, 2013)
Spp1	Secreted phosphoprotein 1	Atherosclerosis	aorta	aorta	(Shao *et al.*, 2011)
Tnfrsf11b	Tumor necrosis factor receptor superfamily, member 11b	HTC1	UNK	aorta	(Fantauzzo *et al.*, 2008),(Callegari *et al.*, 2013),(Yun *et al.*, 2001)
Trim24	Tripartite motif-containing 24	UNK	UNK	skin, muzzle skin, eye, heart, aorta, kidney, lung, tongue	(Ignat *et al.*, 2008)

‡ PXE, pseudoxanthoma elasticum; HOPS, hypophosphatasia, childhood and hypophosphatasia, infantile; CMDD, craniometaphyseal dysplasia, autosomal dominant; HHC1, hypocalciuric hypercalcemia, familial, type 1; GACI, generalized arterial calcification of infancy; HFTC, hyperphosphatemic familial tumoral calcinosis; PXE/VKCFD1, PXE-like disorder with multiple coagulation factor deficiency; KTLS, Keutel syndrome; ACDC, arterial calcification due to CD73 deficiency; NFTC, normophosphatemic tumoral calcinosis; IBGC1, idiopathic basal ganglia calcification 1; DISH, diffuse idiopathic skeletal hyperostosis; HTC1, hypertrichosis universalis congenita, Ambras type. UNK, unknown.

Table S2. Numbers of mice evaluated by sex and strain/cross for phenotype and/or genotyping

Strain/Cross*	Phenotyping Tissues			DNA Collected for GigaMuga Arrays		
	Females	Males	Total	Females	Males	Total
C57BL/6J	14	9	23	1	1	2
DBA/2J	11	10	21	1	1	2
KK/HlJ	5	5	10	1	1	2
B6KKF1	12	9	21	1	1	2
D2KKF1	10	9	19	1	1	1
(B6KKF1)KK	91	92	183	91	92	183
(D2KKF1)KK	97	94	191	97	94	191

*strain/cross designations assigned by the International Mouse Genetic Nomenclature Committee (http://www.informatics.jax.org/mgihome/nomen/strains.shtml

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SUPPLEMENTARY FIGURE LEGEND

Figure S1. Linear regression analysis of the correlation between the calcium content and the mineralization score in the parental strains and the F1 hybrid mice.

For the number of mice analyzed, see Table S2.

Figure S2. Histopathology of lung mineralization and QTL plots.

(a), Phenotypic scores of the parental strains of mice, F1 and N2 progeny determined by histopathology. Each dot represents a mouse (red, females; blue, males). (b-F), Histopathologic evaluation of ectopic mineralization. Normal lungs were infrequent with mineralization (b, score 0). Most of mice had some minor form of mineralization in their lungs. These consisted of purple crystalline structures within alveoli (c, score 1, arrow), to amorphous purple structures within the alveolar wall (d, score 1, arrow). The few moderately affected mice had multiple crystalline purple structures that often fragmented as a sectioning artifact. These were larger than the occasional mild forms (e, score 2). f, enlarged boxed area in e. Ectopic mineralization is indicated by arrows. Scale bar – 100 μm. (g-h), QTL plots from KK x B6 and KK x D2 crosses. KK and D2 both have the *Abcc6* mutant allele while B6 has the wild type allele. Therefore, in KK x B6 cross, a major QTL was identified on Chr. 7 that involves *Abcc6* ($P < 0.05$); however, there is no linkage to *Abcc6* in KK x D2 cross.

Figure S3. Histopathology of eye mineralization and QTL plots.

(a), Phenotypic scores of the parental strains of mice, F1 and N2 progeny determined by histopathology. Each dot represents a mouse (red, females; blue, males). (b-o), Histopathologic evaluation of ectopic mineralization. Corneal stroma mineralization developed to various degrees within the corneal stroma (b-i, score 0 to score 3). Early mild lesions appeared to involve the

Decemet's membrane but periodic acid shiff (PAS) staining (basement membrane) demonstrated mineralization was in the stroma (j, k). Von Kossa (l, m) and Alizarin Red (n, o) confirm the crystalline material is mineralization. Ectopic mineralization is indicated by arrows. Scale bar = 100 μm (panels b, d, f, h, j, l, and n). Scale bar = 10 μm (panels c, e, g, I, k, m, and o). (p-q), QTL plots from KK x B6 and KK x D2 crosses. KK and D2 both have the *Abcc6* mutant allele while B6 has the wild type allele. Therefore, in KK x B6 cross, a major QTL was identified on Chr. 7 that involves *Abcc6* ($P < 0.05$); however, there is no linkage to *Abcc6* in KK x D2 cross.

Figure S4. Demonstration of *Car2* and *Postn* as candidate genes in Chr.3 QTL using GeneWeaver.

A hierarchical similarity chart of ectopic mineralization-related gene sets. At the bottom of the chart are individual gene sets derived from QTL studies, existing cardiac transcriptome data, Sanger SNP database, and String database. Each successive level from the bottom to the top of the chart represents increasingly higher order intersections among these gene sets, with the most highly connected genes at the top.

Figure S5. Demonstration of *Abcg3, Chek2, Aldh2, Ttc28, Hnf1a, Idua,* and *Dmp1* as candidate genes in Chr.5 QTL using GeneWeaver.

A hierarchical similarity chart of ectopic mineralization-related gene sets. At the bottom of the chart are individual gene sets derived from QTL studies, existing kidney transcriptome data, Sanger SNP database, and String database. Each successive level from the bottom to the top of the chart represents increasingly higher order intersections among these gene sets, with the most highly connected genes at the top.