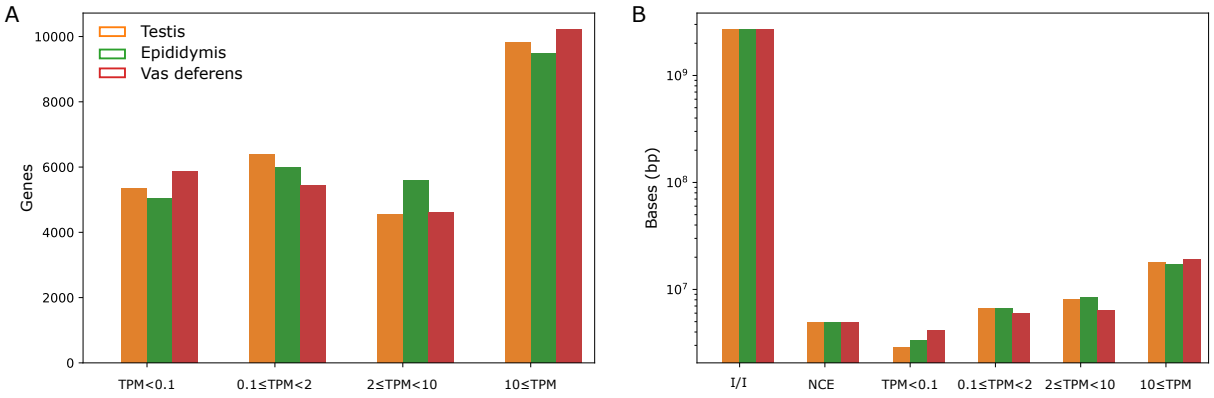
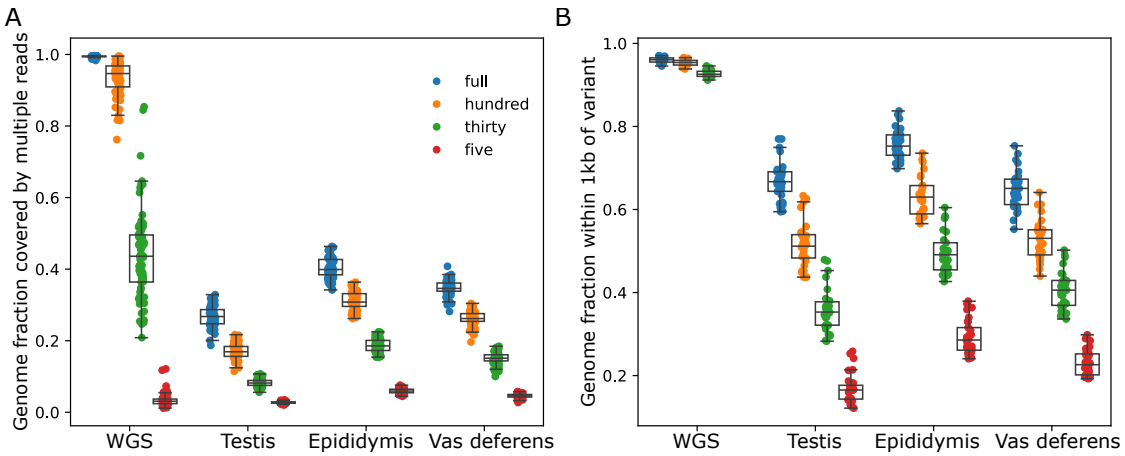
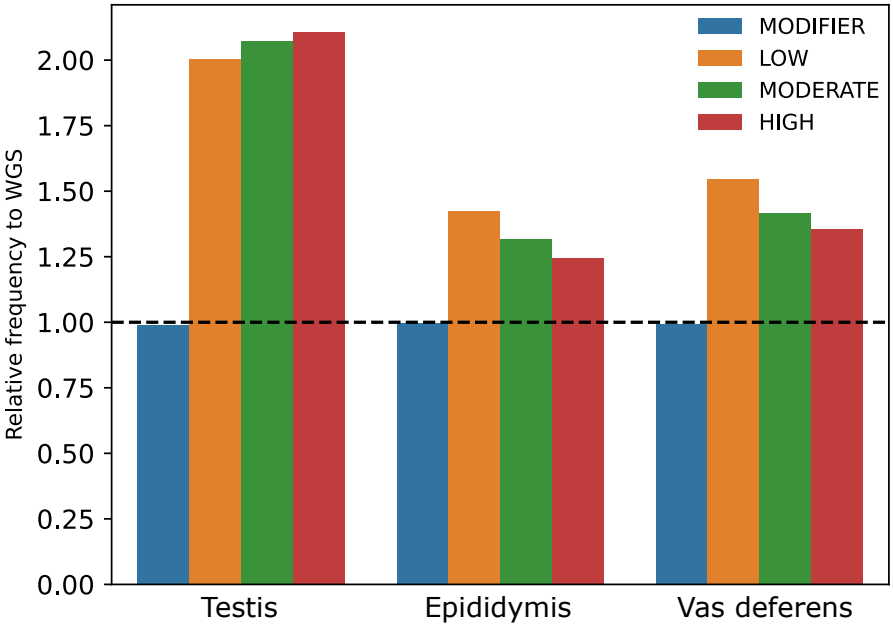


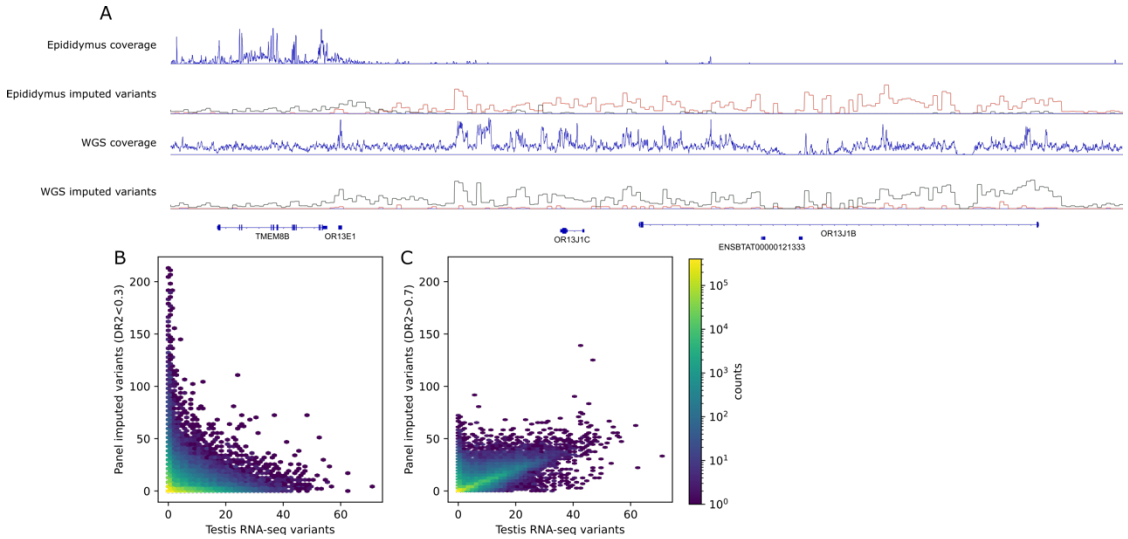
*Supplementary Figure 1. (**A**) Number of genes expressed at different TPM thresholds. (**B**) Total number of bases classified into different regions, for intergenic/intronic (I/I), noncoding exons (NCE), and the different TPM thresholds for coding exons.*



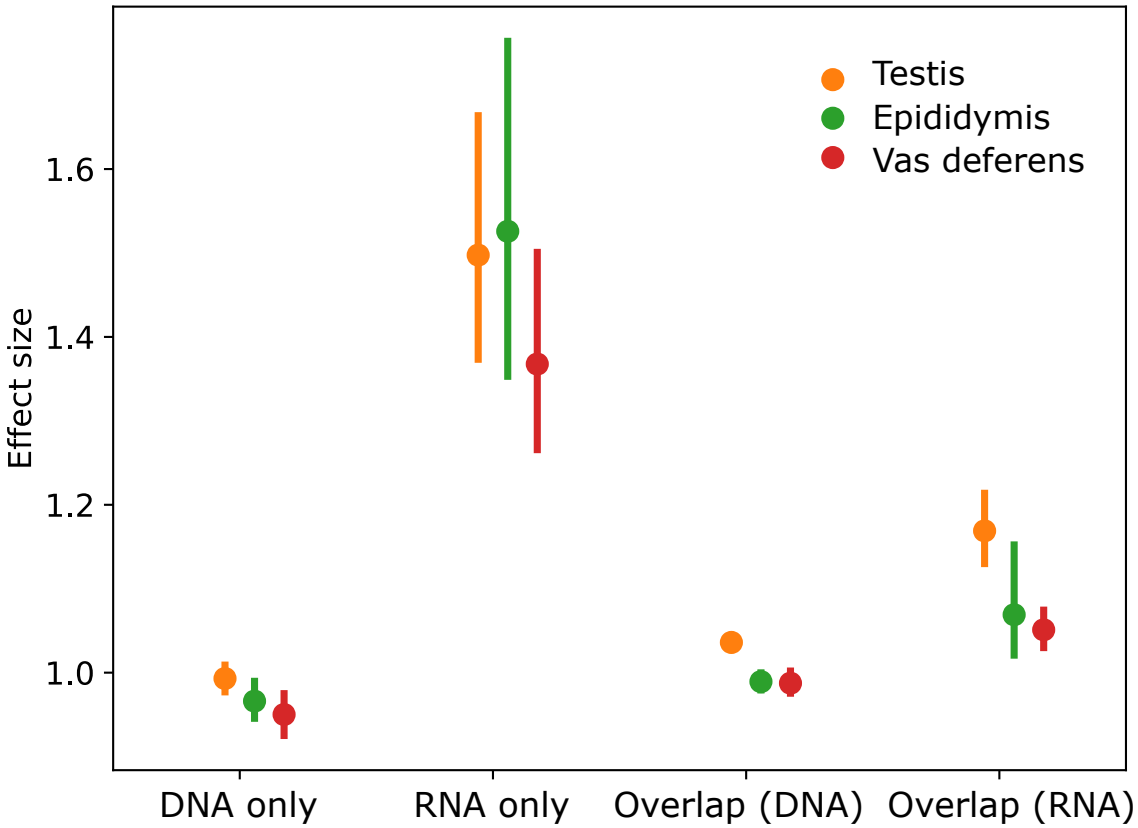
*Supplementary Figure 2. (**A**) Fraction of autosomes covered by at least two reads at different levels of subsampling. (**B**) Fraction of autosomal sequence with at least one variant within 1 Kb at different levels of subsampling.*



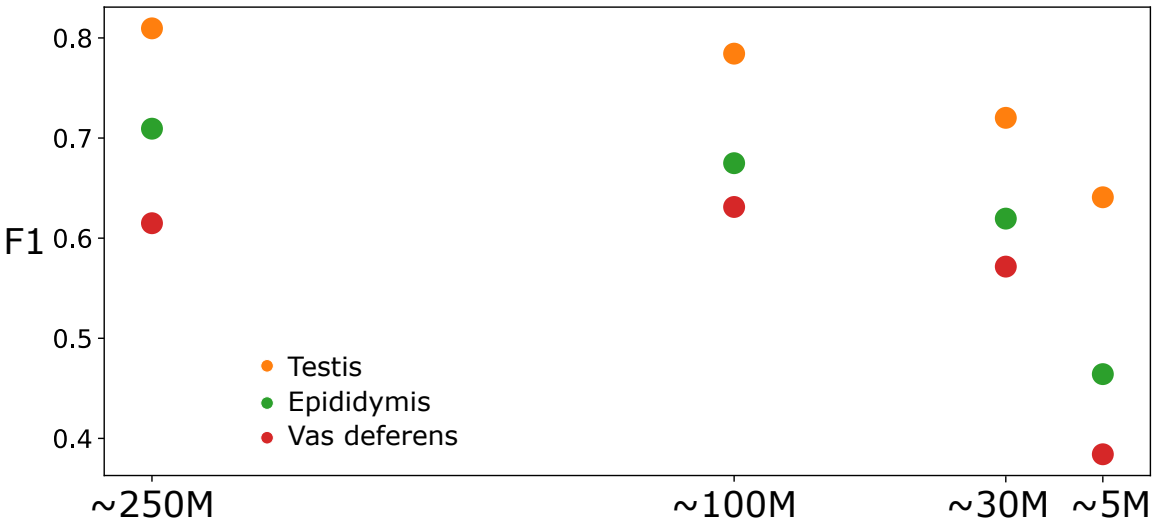
Supplementary Figure 3. Relative frequency of Variant Effect Predictor "IMPACT" feature normalised per impact feature relative to WGS.



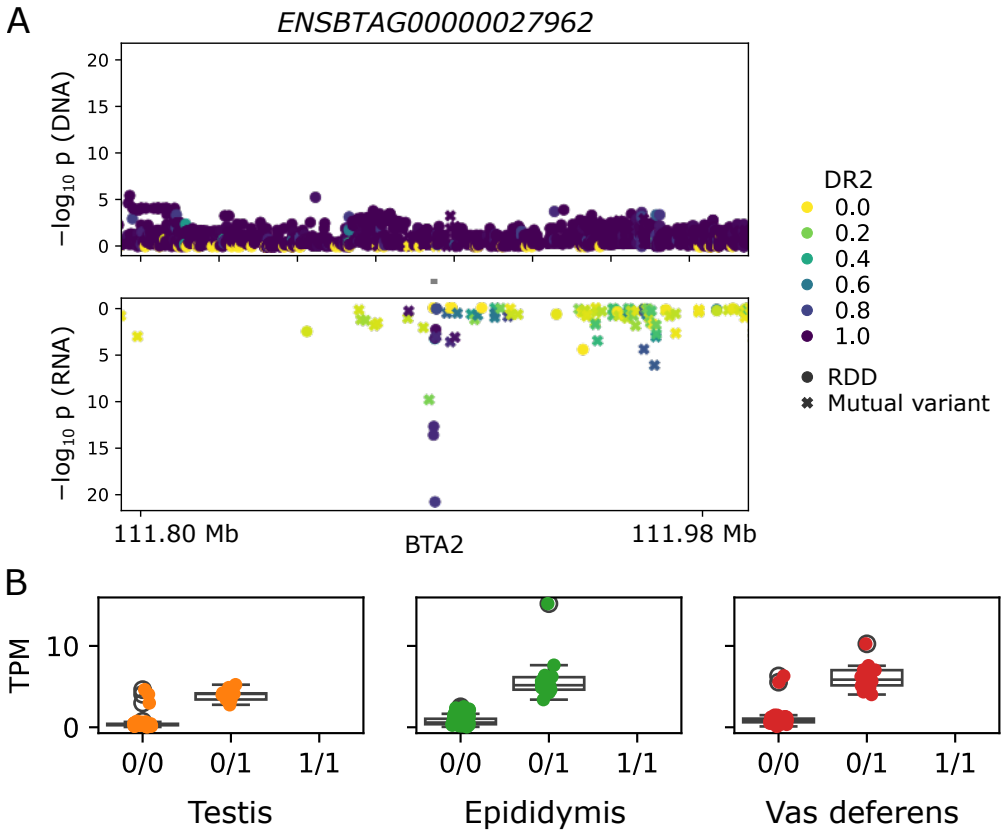
*Supplementary Figure 4. (**A**) Regions with sufficient RNA-seq coverage (shown here for epididymis over the 215 Kb span of BTA8:59992826-60207597) correspond to regions of accurately imputed reference panel variants (black), whereas regions with low RNA-seq coverage correspond to regions of poorly imputed reference panel variants (red). DNA-seq, with more even coverage, tends to also have an even distribution of accurately imputed variants. (**B**) and (**C**) respectively shown this correlation autosome-wide, with the number of variants called from RNA-seq (shown here for testis) in 1 Kb bins against the number of poorly or accurately imputed reference panel variants. Reference markers with imputed frequency of zero were filtered out, which would further inflate the number of poorly imputed variants when there is no RNA-seq coverage nearby.*



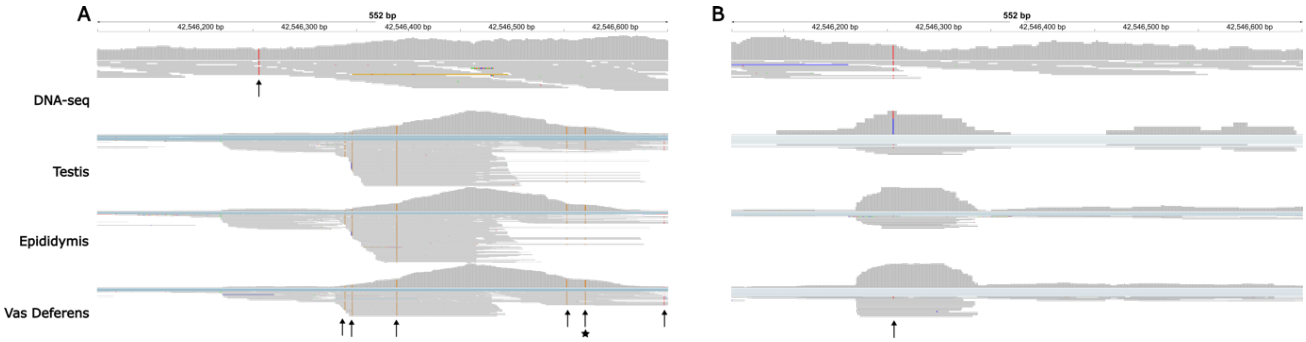
Supplementary Figure 5. Absolute values for effect size for eQTL in three tissues for eQTL found uniquely with DNA variants or RNA variants, as well as common eQTL.



Supplementary Figure 6. F1 score taking DNA-variant eGenes as truth. Molecular phenotypes are also calculated from the subsampled RNA coverage, and so the number of eGenes in the truth set also shrinks at lower coverage.



*Supplementary Figure 7. (**A**) Zoom plots for an eGene associated only with an RNA-seq variant. The grey bar between the DNA and RNA associations represents the gene, while the marker colour represents imputation accuracy (DR2). The marker style indicates if the variant is present in both DNA-seq and RNA-seq variants or if it is an RDD. (**B**) TPM plot for the respective gene. The lead variant is an RDD and can only be examined for RNA-seq but is present in all three tissues.*



Supplementary Figure 8. IGV alignments of DNA-seq and RNA-seq for the three tissues surrounding the lead variant for ENSBTAG00000053969 *in a sample with homozygous alternate genotypes (**A**) and homozygous reference genotypes (**B**) over the range 19:42546100-42546650. RDDs are marked by arrows, and the lead eQTL variant is additionally marked with a star. The RNA-seq coverage scales are significantly different, ranging up to 800 aligned reads in (**A**) and up to 70 aligned reads in (**B**).*

Supplementary Table 1. Publicly available DNA-seq and matched RNA-seq in three tissues for 74 Braunvieh cattle samples, with approximately 10-fold coverage for each DNA-seq sample and approximately 250M paired-end reads for the RNA-seq samples.

Sample	DNA	Testis	Epididymis	Vas deferens
BV_1	SAMEA9539940	SAMEA9540537	SAMEA113601208	SAMEA113601309
BV_2	SAMEA9539941	SAMEA9540538	SAMEA113601233	SAMEA113601330
BV_3	SAMEA111328928	SAMEA111328928	SAMEA113601279	SAMEA113601366
BV_4	SAMEA111328929	SAMEA111328929	SAMEA113601280	SAMEA113601367
BV_5	SAMEA111328925	SAMEA111328925	SAMEA113601276	SAMEA113601361
BV_6	SAMEA9539945	SAMEA9540542	SAMEA113601252	SAMEA113601346
BV_7	SAMEA9539946	SAMEA9540543	SAMEA113601232	SAMEA113601329
BV_8	SAMEA9539947	SAMEA9540544	SAMEA113601212	SAMEA113601312
BV_9	SAMEA9539948	SAMEA9540545	SAMEA113601213	SAMEA113601314
BV_10	SAMEA9539949	SAMEA9540546	SAMEA113601215	SAMEA113601316
BV_11	SAMEA111328926	SAMEA111328926	SAMEA113601277	SAMEA113601362
BV_12	SAMEA111328917	SAMEA111328917	SAMEA113601266	SAMEA113601355
BV_13	SAMEA113578975	SAMEA113578975	SAMEA113601290	SAMEA113601375
BV_14	SAMEA9539950	SAMEA9540547	SAMEA113601250	SAMEA113601345
BV_15	SAMEA9539953	SAMEA9540550	SAMEA113601244	SAMEA113601340
BV_16	SAMEA9539954	SAMEA9540551	SAMEA113601220	SAMEA113601317
BV_17	SAMEA9539955	SAMEA9540552	SAMEA113601214	SAMEA113601315
BV_18	SAMEA9539957	SAMEA9540554	SAMEA113601248	SAMEA113601343
BV_19	SAMEA9539961	SAMEA9540558	SAMEA113601205	SAMEA113601306
BV_20	SAMEA9539964	SAMEA9540561	SAMEA113601204	SAMEA113601305

BV_21	SAMEA9539966	SAMEA9540563	SAMEA113601195	SAMEA113601298
BV_22	SAMEA9539967	SAMEA9540564	SAMEA113601241	SAMEA113601337
BV_23	SAMEA9539968	SAMEA9540565	SAMEA113601201	SAMEA113601303
BV_24	SAMEA9539969	SAMEA9540566	SAMEA113601239	SAMEA113601334
BV_25	SAMEA111328924	SAMEA111328924	SAMEA113601275	SAMEA113601360
BV_26	SAMEA111328941	SAMEA111328941	SAMEA113601294	SAMEA113601380
BV_27	SAMEA113578979	SAMEA113578979	SAMEA113601284	SAMEA113601372
BV_28	SAMEA113578980	SAMEA113578980	SAMEA113601291	SAMEA113601377
BV_29	SAMEA9539970	SAMEA9540567	SAMEA113601230	SAMEA113601327
BV_30	SAMEA9539971	SAMEA9540568	SAMEA113601199	SAMEA113601301
BV_31	SAMEA111328930	SAMEA111328930	SAMEA113601282	SAMEA113601369
BV_32	SAMEA113578981	SAMEA113578981	SAMEA113601278	SAMEA113601365
BV_33	SAMEA9539972	SAMEA9540569	SAMEA113601243	SAMEA113601339
BV_34	SAMEA9539974	SAMEA9540571	SAMEA113601240	SAMEA113601336
BV_35	SAMEA9539975	SAMEA9540572	SAMEA113601198	SAMEA113601300
BV_36	SAMEA9539976	SAMEA9540573	SAMEA113601200	SAMEA113601302
BV_37	SAMEA9539977	SAMEA9540574	SAMEA113601229	SAMEA113601326
BV_38	SAMEA9539978	SAMEA9540575	SAMEA113601223	SAMEA113601320
BV_39	SAMEA9539979	SAMEA9540576	SAMEA113601227	SAMEA113601324
BV_40	SAMEA113578967	SAMEA113578967	SAMEA113601272	SAMEA113601358
BV_41	SAMEA111328932	SAMEA111328932	SAMEA113601285	SAMEA113601373
BV_42	SAMEA111328931	SAMEA111328931	SAMEA113601283	SAMEA113601370
BV_43	SAMEA9539980	SAMEA9540577	SAMEA113601231	SAMEA113601328
BV_44	SAMEA9539981	SAMEA9540578	SAMEA113601207	SAMEA113601308
BV_45	SAMEA9539982	SAMEA9540579	SAMEA113601211	SAMEA113601313
BV_46	SAMEA111328916	SAMEA111328916	SAMEA113601265	SAMEA113601354
BV_47	SAMEA111328913	SAMEA111328913	SAMEA113601263	SAMEA113601352
BV_48	SAMEA9539984	SAMEA9540581	SAMEA113601202	SAMEA113601304
BV_49	SAMEA9539986	SAMEA9540583	SAMEA113601210	SAMEA113601311
BV_50	SAMEA113578969	SAMEA113578969	SAMEA113601281	SAMEA113601368
BV_51	SAMEA9539989	SAMEA9540586	SAMEA113601242	SAMEA113601338
BV_52	SAMEA9539990	SAMEA9540587	SAMEA113601197	SAMEA113601299
BV_53	SAMEA9539991	SAMEA9540588	SAMEA113601221	SAMEA113601318
BV_54	SAMEA9539992	SAMEA9540589	SAMEA113601225	SAMEA113601322
BV_55	SAMEA9539993	SAMEA9540590	SAMEA113601226	SAMEA113601323
BV_56	SAMEA111328912	SAMEA111328912	SAMEA113601262	SAMEA113601351
BV_57	SAMEA9539999	SAMEA9540596	SAMEA113601209	SAMEA113601310
BV_58	SAMEA9540000	SAMEA9540597	SAMEA113601228	SAMEA113601325
BV_59	SAMEA9540005	SAMEA9540602	SAMEA113601249	SAMEA113601344
BV_60	SAMEA9540002	SAMEA9540599	SAMEA113601247	SAMEA113601342
BV_61	SAMEA113578970	SAMEA113578970	SAMEA113601289	SAMEA113601374
BV_62	SAMEA9540001	SAMEA9540598	SAMEA113601224	SAMEA113601321
BV_63	SAMEA111328940	SAMEA111328940	SAMEA113601293	SAMEA113601379

BV_64	SAMEA9540003	SAMEA9540600	SAMEA113601245	SAMEA113601341
BV_65	SAMEA9540007	SAMEA9540604	SAMEA113601255	SAMEA113601348
BV_66	SAMEA9540008	SAMEA9540605	SAMEA113601253	SAMEA113601347
BV_67	SAMEA9540009	SAMEA9540606	SAMEA113601238	SAMEA113601333
BV_68	SAMEA113578971	SAMEA113578971	SAMEA113601273	SAMEA113601359
BV_69	SAMEA9540011	SAMEA9540608	SAMEA113601237	SAMEA113601332
BV_70	SAMEA111328920	SAMEA111328920	SAMEA113601269	SAMEA113601356
BV_71	SAMEA111328922	SAMEA111328922	SAMEA113601271	SAMEA113601357
BV_72	SAMEA9540013	SAMEA9540610	SAMEA113601257	SAMEA113601349
BV_73	SAMEA9540015	SAMEA9540612	SAMEA113601235	SAMEA113601331
BV_74	SAMEA111328942	SAMEA111328942	SAMEA113601295	SAMEA113601381

Supplementary Table 2. Number of expressed genes passing filtering thresholds for the three RNA tissues at full coverage (~250 M reads) as well as the three downsampled coverages. The number of significant eGenes (using DNA-seq variants) is given in parentheses.

	Full coverage	100 M reads	30 M reads	5 M reads
Testis	20,960 (7,377)	20,169 (6,229)	18,563 (4,220)	15,867 (1,264)
Epididymis	21,271 (3,084)	20,260 (2,443)	18,423 (1,227)	15,272 (234)
Vas deferens	20,097 (1,962)	19,032 (1,473)	17,065 (965)	13,936 (239)

*Supplementary Table 3. eGenes uniquely detected with RNA-seq and not with DNA-seq as the source of genomic variants. Many of the eGenes have substantial number of paralogues or pseudogenes, leading to higher likelihood of mis/multi-mapping reads. Tissues indicated with * mean the eGene was uniquely found using RNA-seq variants, but the significance was not below 1×10^{-10}. Top associated variants are indicated as RNA-DNA differences (RDD) or not.*

Gene	Gene type	paralogues	Tissues	RDD
ENSBTAG00000001858	Protein coding	5	T, E, V	Y
ENSBTAG00000007816	Processed pseudogene	3	E, V	Y
ENSBTAG00000009110	Pseudogene	1	T*, E*, V	Y
ENSBTAG00000012798 (KCNH8)	Protein coding	17	E*, V	N
ENSBTAG00000020620 (RGS2)	Protein coding	25	T*, E*, V	Y
ENSBTAG00000021565 (PRSS2)	Protein coding	3	T	N
ENSBTAG00000027962	Protein coding	1	T, E, V	Y
ENSBTAG00000038064	Protein coding	5	T, E	N
ENSBTAG00000040518	Protein coding	22	T*, E, V*	Y
ENSBTAG00000042358	snoRNA	16	T*, E, V*	Y
ENSBTAG00000043349	snoRNA	16	T, E, V*	Y
ENSBTAG00000050214	Processed pseudogene	6	T, E*, V*	Y
ENSBTAG00000053419	Protein coding	2	T*, E, V*	Y
ENSBTAG00000053570	Processed pseudogene	22	T*, E, V	Y
ENSBTAG00000053969	Protein coding	21	T, E, V	Y

Supplementary Table 4. Median genotype quality and imputation accuracy (DR2) for variants called in both DNA- and RNA-seq, called only in RNA-seq, and RNA-seq variants that were significantly associated with RNA-only eGenes.

RNA-DNA overlap		RNA-DNA differences		RNA-only eQTL	
Genotype quality	Imputation accuracy	Genotype quality	Imputation accuracy	Genotype quality	Imputation accuracy

WGS	63	1.00	-	-	-	-
Testis	30	0.81	19	0.63	32	0.91
Epididymis	36	0.98	22	0.67	32	0.87
Vas deferens	35	0.97	22	0.66	33	0.85