Supplementary Table 1. Study population characteristics stratified by number of heteroplasmies.

	Multi-Ethnic Study of Arteriosclerosis			**Rotterdam Study**		
	0 (N=643)	**1+ (N=388)**	**Overall (N=1031)**	**0 (N=221)**	**1+ (N=152)**	**Overall (N=373)**
Age (Time 1)						
Mean (SD)	58.7 (9.0)	60.1 (8.8)	59.2 (8.9)	64.5 (5.54)	66.7 (6.16)	65.4 (5.89)
Median [Min, Max]	58.0 [44.0, 83.0]	59.0 [45.0, 82.0]	58.0 [44.0, 83.0]	64.3 [55.4, 79.9]	65.6 [55.4, 95.1]	64.8 [55.4, 95.1]
Sex						
Women	350 (54.4%)	204 (52.6%)	554 (53.7%)	123 (55.7%)	94 (61.8%)	217 (58.2%)
Men	293 (45.6%)	184 (47.4%)	477 (46.3%)	98 (44.3%)	58 (38.2%)	156 (41.8%)
Race						
White / European	248 (38.6%)	176 (45.4%)	424 (41.1%)	217 (98.2%)	150 (98.7%)	367 (98.4%)
Chinese / Asian	19 (3.0%)	9 (2.3%)	28 (2.7%)	2 (0.9%)	2 (1.3%)	4 (1.1%)
Hispanic	229 (35.6%)	118 (30.4%)	347 (33.7%)			
Black / African	147 (22.9%)	85 (21.9%)	232 (22.5%)	2 (0.9%)	0 (0%)	2 (0.5%)
Smoking (Time 1)						
Current	79 (12.3%)	62 (16.0%)	141 (13.7%)	47 (21.3%)	33 (21.7%)	80 (21.4%)

Never	313 (48.7%)	175 (45.1%)	488 (47.3%)	63 (28.5%)	47 (30.9%)	110 (29.5%)
Previous	250 (38.9%)	151 (38.9%)	401 (38.9%)	107 (48.4%)	71 (46.7%)	178 (47.7%)
Missing	1 (0.2%)		1 (0.1%)	4 (1.8%)	1 (0.7%)	5 (1.3%)
Smoking (Time 2)						
Current	53 (8.2%)	39 (10.1%)	92 (8.9%)	48 (21.7%)	28 (18.4%)	76 (20.4%)
Never	268 (41.7%)	152 (39.2%)	420 (40.7%)	64 (29.0%)	48 (31.6%)	112 (30.0%)
Previous	316 (49.1%)	196 (50.5%)	512 (49.7%)	109 (49.3%)	76 (50.0%)	185 (49.6%)
Missing	6 (0.9%)	1 (0.3%)	7 (0.7%)			
Time Between Visits (yrs)						
Mean (SD)	9.39 (0.40)	9.39 (0.37)	9.39 (0.39)	6.45 (0.327)	6.43 (0.324)	6.44 (0.325)
Median [Min, Max]	9.42 [8.10,10.60]	9.41 [7.96, 10.50]	9.42 [7.96, 10.60]	6.42 [5.29, 8.32]	6.40 [5.17, 7.80]	6.41 [5.17, 8.32]
Status						
Alive	579 (90.0%)	335 (86.3%)	914 (88.7%)	146 (66.1%)	101 (66.4%)	247 (66.2%)
Dead	63 (9.8%)	53 (13.7%)	116 (11.3%)	75 (33.9%)	51 (33.6%)	126 (33.8%)
Missing	1 (0.2%)		1 (0.1%)			

Time to Event (yrs)						
Mean (SD)	7.75 (1.30)	7.65 (1.45)	7.71 (1.36)	8.57 (2.55)	8.58 (2.60)	8.57 (2.57)
Median [Min, Max]	8.07 [0.972, 8.68]	8.07 [1.08, 8.68]	8.07 [0.972, 8.68]	10.0 [0.110, 10.0]	10.0 [0.244, 10.0]	10.0 [0.110, 10.0]
Missing	1 (0.2%)	2 (0.5%)	3 (0.3%)			

Supplementary Table 2. deltaVAF stratified by extant vs. de novo

	Multi-Ethnic Study of Atherosclerosis		**Rotterdam Study**	
deltaVAF	**Extant (385)**	**De novo (126)**	**Extant (172)**	**De novo (39)**
-0.10	22	0	5	0
-0.05	38	0	14	0
Neutral	305	20	126	7
+0.05	17	27	12	7
+0.10	12	48	13	19
+0.20	0	15	2	6

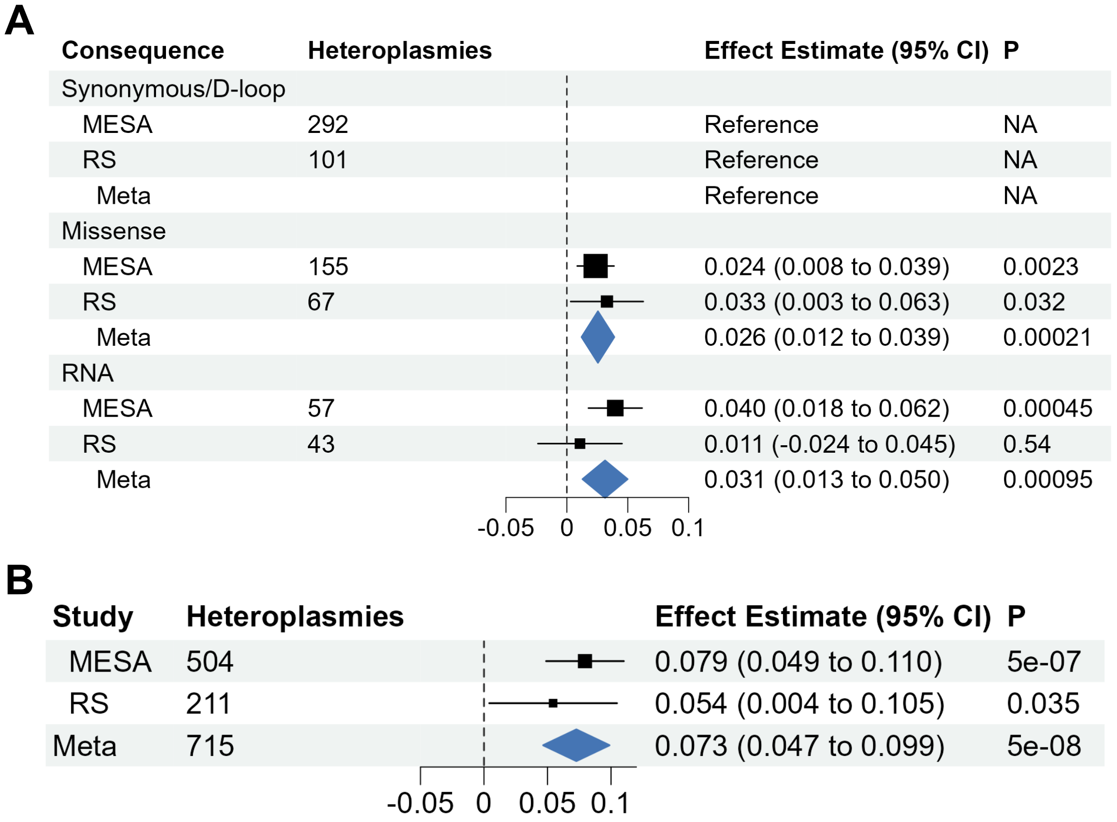
Note: Negative values are less or equal positive values are greater or equal

Supplementary Table 3. Associations between rank-transformed deltaVAF and all-cause mortality

	n/N	**MESA** **HR (95% CI)**	**n/N**	**RS** **HR (95% CI)**
Model 1	53/385	1.46 (1.06, 1.99)	51/152	1.38 (1.04, 1.82)

Note: Model 1 was adjusted for age using a natural spline with 4 degrees of freedom, sex, race, and smoking status (current, previous, never). Analyses for MESA also included DNA collection center and sequencing batch.

Supplementary Fig 1. Meta-analyses of association between functional categories and functional consequence and deltaVAF.

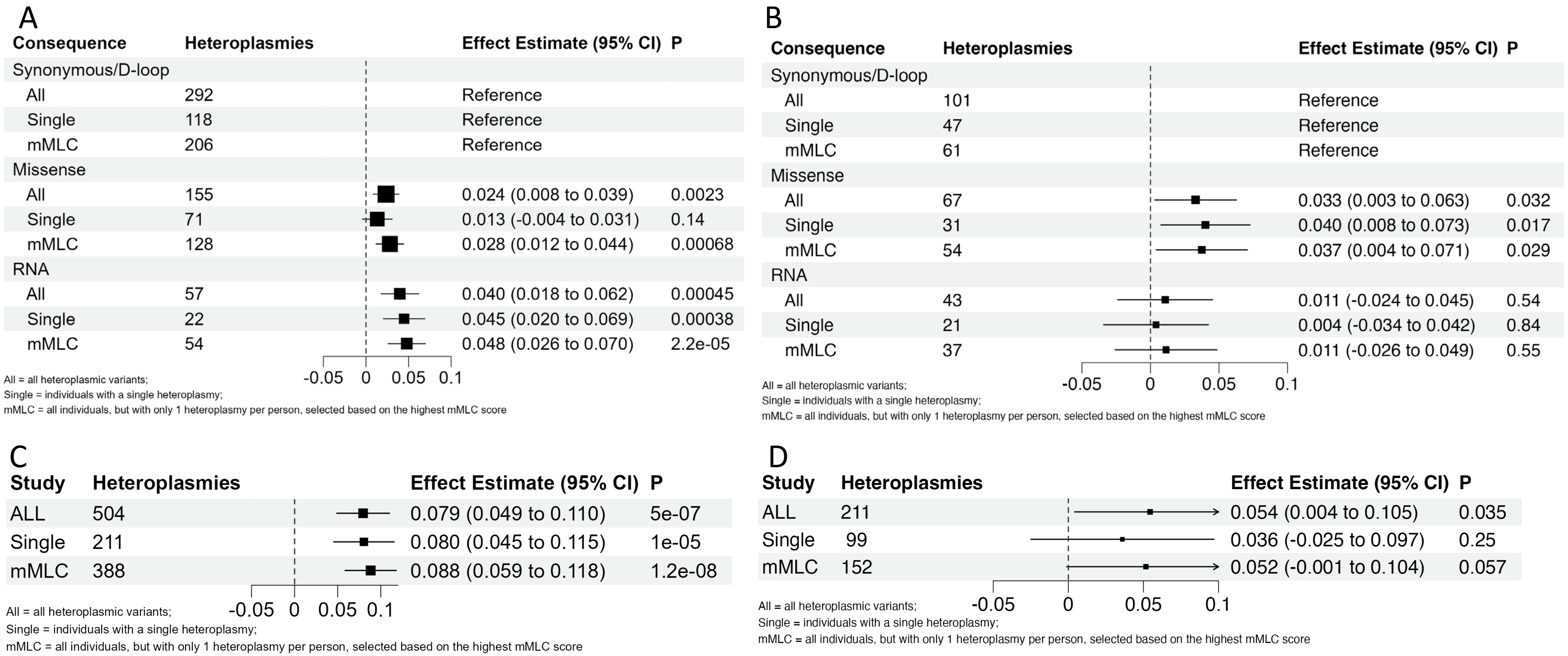


Consequence	Heteroplasmies	Effect Estimate (95% CI)	P
Synonymous/D-loop			
MESA	292	Reference	NA
RS	101	Reference	NA
Meta		Reference	NA
Missense			
MESA	155	0.024 (0.008 to 0.039)	0.0023
RS	67	0.033 (0.003 to 0.063)	0.032
Meta		0.026 (0.012 to 0.039)	0.00021
RNA			
MESA	57	0.040 (0.018 to 0.062)	0.00045
RS	43	0.011 (-0.024 to 0.045)	0.54
Meta		0.031 (0.013 to 0.050)	0.00095

Study	Heteroplasmies	Effect Estimate (95% CI)	P
MESA	504	0.079 (0.049 to 0.110)	5e-07
RS	211	0.054 (0.004 to 0.105)	0.035
Meta	715	0.073 (0.047 to 0.099)	5e-08

a, Meta-analyses of the associations of functional categories on deltaVAF. **b**, Meta-analysis of mMLC on deltaVAF

Supplementary Fig 2. Association between functional categories and functional consequence and deltaVAF in sub-sampled cohorts.



A

Consequence	Heteroplasmies	Effect Estimate (95% CI)	P
Synonymous/D-loop			
All	292	Reference	
Single	118	Reference	
mMLC	206	Reference	
Missense			
All	155	0.024 (0.008 to 0.039)	0.0023
Single	71	0.013 (-0.004 to 0.031)	0.14
mMLC	128	0.028 (0.012 to 0.044)	0.00068
RNA			
All	57	0.040 (0.018 to 0.062)	0.00045
Single	22	0.045 (0.020 to 0.069)	0.00038
mMLC	54	0.048 (0.026 to 0.070)	2.2e-05

All = all heteroplasmic variants;
Single = individuals with a single heteroplasmy;
mMLC = all individuals, but with only 1 heteroplasmy per person, selected based on the highest mMLC score

B

Consequence	Heteroplasmies	Effect Estimate (95% CI)	P
Synonymous/D-loop			
All	101	Reference	
Single	47	Reference	
mMLC	61	Reference	
Missense			
All	67	0.033 (0.003 to 0.063)	0.032
Single	31	0.040 (0.008 to 0.073)	0.017
mMLC	54	0.037 (0.004 to 0.071)	0.029
RNA			
All	43	0.011 (-0.024 to 0.045)	0.54
Single	21	0.004 (-0.034 to 0.042)	0.84
mMLC	37	0.011 (-0.026 to 0.049)	0.55

All = all heteroplasmic variants;
Single = individuals with a single heteroplasmy;
mMLC = all individuals, but with only 1 heteroplasmy per person, selected based on the highest mMLC score

C

Study	Heteroplasmies	Effect Estimate (95% CI)	P
ALL	504	0.079 (0.049 to 0.110)	5e-07
Single	211	0.080 (0.045 to 0.115)	1e-05
mMLC	388	0.088 (0.059 to 0.118)	1.2e-08

All = all heteroplasmic variants;
Single = individuals with a single heteroplasmy;
mMLC = all individuals, but with only 1 heteroplasmy per person, selected based on the highest mMLC score

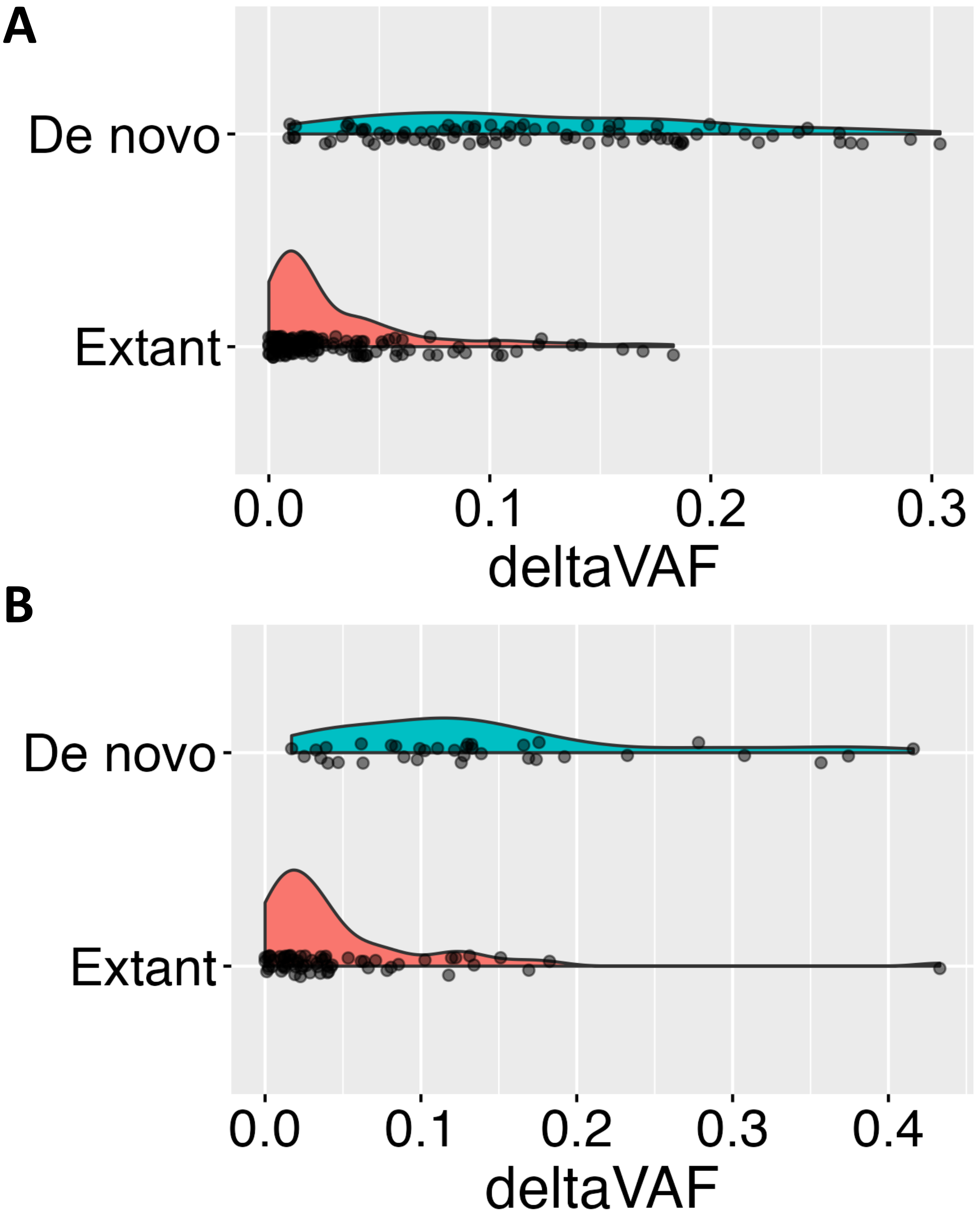
D

Study	Heteroplasmies	Effect Estimate (95% CI)	P
ALL	211	0.054 (0.004 to 0.105)	0.035
Single	99	0.036 (-0.025 to 0.097)	0.25
mMLC	152	0.052 (-0.001 to 0.104)	0.057

All = all heteroplasmic variants;
Single = individuals with a single heteroplasmy;
mMLC = all individuals, but with only 1 heteroplasmy per person, selected based on the highest mMLC score

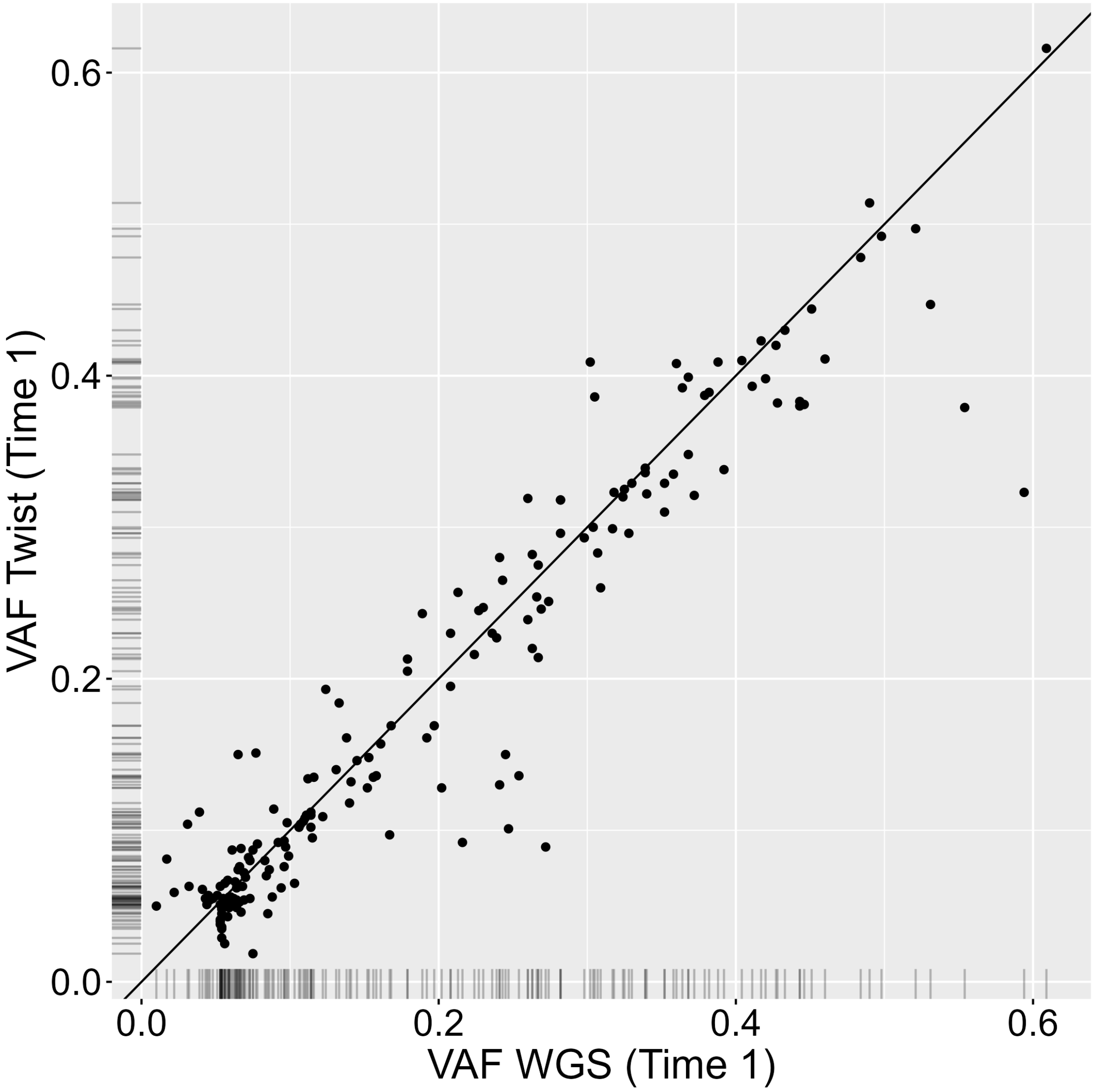
a,b, Associations of functional categories with deltaVAF in (**a**) MESA and (**b**) RS. c,d, Association of mMLC and deltaVAF in (**c**) MESA and (**d**) RS. ALL indicates the full cohort, Single indicates individuals with only a single heteroplasmy, and mMLC indicates all individuals, but with only 1 heteroplamsy chosen per person, selected based on the highest mMLC score.

Supplementary Fig 3. deltaVAF distribution stratified by de novo status.



a,**b**, Density plots of deltaVAF stratified by de novo status in in (**a**) MESA and (**b**) RS. De novo is defined is being VAF<0.05 at baseline, and greater than 0.05 in the second visit.

Supplementary Fig 4. Comparison of VAF between WGS and Twist sequencing.



Correlation between VAF at the first measurement (x-axis) and the second measurement (y-axis), with the black line indicating perfect correlation. Data is generated from 406 MESA samples sequenced by both technologies at the baseline visit.