Supplemental Data

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Table S7: Association of plasma biomarkers with annual change in eGFR (ml/min/1.73m^2) in staged linear mixed effects models. β (95% CIs) for change in annual eGFR slope per unit log_2-transformed plasma biomarker concentration within the subcohort (N=597)

Table S8: BioPETsurv analysis of modest to highly prognostic biomarkers for a 5-year clinical trial of DKD progression.

Figure S1: Flow Diagram of Sample Selection

Figure S2: Scatterplot of plasma biomarkers (log_2-transformed) on y-axis by baseline eGFR (ml/min/1.73m^2) on x-axis. Top row (R-L): KIM-1, TNFR-1, TNFR-2. Bottom row (R-L): MCP-1, suPAR, YKL-40.

Figure S3: BioPETsurv analysis of KIM-1 as a modest prognostic biomarker for a 5-year clinical trial of DKD progression.

Figure S4: BioPETsurv analysis of TNFR-2 as a highly prognostic biomarker for a 5-year clinical trial of DKD progression.

Table S1: Quality control parameters of biospecimen assays.

Assay	Intra-assay*		Inter-assay^		Lower Limit of Detection (pg/ml)	Upper Limit of Detection (pg/ml)
	Mean CV, %	CV% Range	Mean CV, %	CV% Range		
KIM-1	4.3	0 – 21.5	10.0	0.1 - 49.6	1.98	20,000
TNFR-1	7.0	0 – 124.6	14.8	0.5 - 74.1	0.67	16,000
TNFR-2	4.1	0 - 41.4	11.0	0.1 - 51.4	0.17	20,000
MCP-1	3.7	0 - 75	11.2	0.9 - 42.6	0.31	3,900
suPAR	5.4	0 – 129.1	15.3	1.9 - 49.8	53	64,000
YKL-40	2.9	0 - 31.1	9.9	0.2 - 52.9	140	192,000

CV – coefficient of variation; KIM-1- kidney injury molecule-1; MCP-1: Monocyte chemotactic protein; TNFR-1: tumor necrosis factor 1; TNFR-2: tumor necrosis factor 2; suPAR: soluble urokinase-type plasminogen activator receptor

*CV represents variability between duplicate pairs on assayed on the same day (N=894)

^CV represents variability between blind duplicates assayed on different days (N=45)

Table S2. Plasma values of biomarkers according to baseline eGFR category (n=894)

	eGFR <30 (N=335)	**eGFR ≥30 to <45 (N=335)**	**eGFR 45 to <60 (N=224)**	*P**
suPAR (pg/mL)	10,949 (8,784 - 13,796)	7,451 (5,811 - 9,420)	5,599 (4,273 - 6,764)	<0.001
TNFR-1 (pg/mL)	6,391 (4,755 - 8,788)	3,654 (2,628 – 4,861)	2,255 (1,724 - 2,957)	<0.001
TNFR-2 (pg/mL)	62,696 (51,083 – 78,210)	43,033 (34,419 – 52,287)	31,223 (25,277 – 37,547)	<0.001
KIM-1 (pg/mL)	983 (533 – 2,176)	659 (381 – 1,188)	457 (268 - 847)	<0.001
MCP-1 (pg/mL)	156 (119 - 188)	135 (108 - 168)	118 (95 – 154)	<0.001
YKL-40 (pg/mL)	199,091 (127,405 – 230,691)	142,633 (87,687 – 215,445)	97,935 (56,109 – 175,842)	<0.001

Data reported as median and interquartile range. eGFR: estimated glomerular filtration rate (mL/min/1.73m^2); suPAR: soluble urokinase-type plasminogen activator receptor; TNFR-1: tumor necrosis factor-1; TNFR-2: tumor necrosis factor-2; KIM-1- kidney injury molecule-1; MCP-1: Monocyte chemotactic protein-1;
*Assessed with ANOVA or Kruskal-Wallis tests as appropriate.

Table S3: Association of plasma biomarkers with risk of DKD progression in staged weighted Cox proportional hazards regression models. HRs (95% CIs) for DKD progression per unit of log_2-transformed plasma biomarker concentration.

Biomarker*	**Model 1** (eGFR)	**Model 2** (UPCR)	**Model 3** (eGFR + UPCR)
KIM-1	1.58 (1.44-1.73)	1.33 (1.19-1.48)	1.26 (1.14-1.40)
TNFR-1	2.23 (1.76-2.82)	2.41 (2.05-2.84)	1.84 (1.45-2.33)
TNFR-2	2.79 (2.06-3.77)	3.23 (2.51-4.16)	2.18 (1.59-3.00)
MCP-1	1.38 (1.12-1.70)	1.54 (1.25-1.90)	1.44 (1.17-1.77)
suPAR	1.55 (1.28-1.87)	1.95 (1.58-2.40)	1.40 (1.14-1.72)
YKL-40	1.47 (1.27-1.70)	1.47 (1.27-1.71)	1.35 (1.16-1.57)

KIM-1- kidney injury molecule-1; MCP-1: Monocyte chemotactic protein; TNFR-1: tumor necrosis factor 1; TNFR2: tumor necrosis factor 2; suPAR: soluble urokinase-type plasminogen activator receptor; eGFR: estimated glomerular filtration rate (mL/min/1.73m^2); UPCR: urine-to-protein creatinine ratio

Base covariates for all models: age, sex, race/ethnicity, education, clinical center, systolic blood pressure, diastolic blood pressure, body mass index, hsCRP, hemoglobin A1c, anti-hypertensive medication use, smoking status

Model 1: base covariates plus baseline eGFR

Model 2: base covariates plus UPCR

Model 3: base covariates plus baseline eGFR and UPCR

*per log_2-transformed biomarkers

Table S4: Association of plasma biomarkers with risk of DKD progression using ComBat approach. HRs (95% CIs) for DKD progression per unit of log_2-transformed plasma biomarker concentration.

Biomarker*	HR (95% CI) (Corrected for mean shift)	HR (95% CI) (Corrected for mean and variance shifts)	HR (95% CI) (Corrected for mean shift of quality control samples)
KIM-1	1.30 (1.17-1.44)	1.33 (1.20-1.47)	1.28 (1.16-1.41)
TNFR-1	1.91 (1.49-2.45)	1.83 (1.45-2.32)	1.92 (1.51-2.44)
TNFR-2	2.46 (1.78-3.41)	2.38 (1.75-3.24)	2.59 (1.94-3.45)
MCP-1	1.54 (1.25-1.90)	1.59 (1.29-1.96)	1.56 (1.27-1.92)
suPAR	1.51 (1.22-1.88)	1.57 (1.27-1.94)	1.54 (1.25-1.89)
YKL-40	1.36 (1.17-1.59)	1.39 (1.19-1.62)	1.37(1.17-1.60)

KIM-1- kidney injury molecule-1; MCP-1: Monocyte chemotactic protein; TNFR-1: tumor necrosis factor 1; TNFR2: tumor necrosis factor 2; suPAR: soluble urokinase-type plasminogen activator receptor; eGFR: estimated glomerular filtration rate (mL/min/1.73m^2); UPCR: urine-to-protein creatinine ratio

Model 1: adjusted for age, sex, race/ethnicity, education, clinical center, systolic blood pressure, diastolic blood pressure, body mass index, hsCRP, hemoglobin A1c, anti-hypertensive medication use, smoking status, UPCR, baseline eGFR

*per 1 unit log_2-transformed biomarkers

Table S5: Association of plasma biomarkers with risk of DKD progression in proportional hazards regression model in the subcohort only. HRs (95% CIs) for DKD progression per unit of log_2-transformed plasma biomarker concentration.

Biomarker*	HR (95% CI)
KIM-1	1.28 (1.12-1.47)
TNFR-1	1.46 (1.07-1.98)
TNFR-2	1.68 (1.16-2.43)
MCP-1	1.38 (1.06-1.80)
suPAR	1.43 (1.02-2.01)
YKL-40	1.30 (1.09-1.56)

KIM-1- kidney injury molecule-1; MCP-1: Monocyte chemotactic protein; TNFR-1: tumor necrosis factor 1; TNFR2: tumor necrosis factor 2; suPAR: soluble urokinase-type plasminogen activator receptor; eGFR: estimated glomerular filtration rate (mL/min/1.73m^2); UPCR: urine-to-protein creatinine ratio
Model adjusted for age, sex, race/ethnicity, education, clinical center, systolic blood pressure, diastolic blood pressure, body mass index, hsCRP, hemoglobin A1c, anti-hypertensive medication use, smoking status, UPCR + baseline eGFR
*per 1 unit log_2-transformed biomarker

Table S6: Association of plasma biomarkers with risk of DKD progression in proportional hazards regression model adjusting for biomarkers. HRs (95% CIs) for DKD progression per unit of log_2-transformed plasma biomarker concentration.

Biomarker*	HR (95% CI)
KIM-1	1.17 (1.05-1.30)
TNFR-2	1.61 (1.15-2.26)
MCP-1	1.20 (0.97-1.47)
YKL-40	1.18 (1.01-1.39)

KIM-1- kidney injury molecule-1; MCP-1: Monocyte chemotactic protein; TNFR-2: tumor necrosis factor 2; eGFR: estimated glomerular filtration rate (mL/min/1.73m^2); UPCR: urine-to-protein creatinine ratio
Model adjusted for age, sex, race/ethnicity, education, clinical center, systolic blood pressure, diastolic blood pressure, body mass index, hsCRP, hemoglobin A1c, anti-hypertensive medication use, smoking status, UPCR + baseline eGFR
*per 1 standard deviation log_2-transformed biomarker
Biomarkers selected to remain in model via backward selection ($p<0.05$)

Table S7: Association of plasma biomarkers with annual change in eGFR (ml/min/1.73m^2) in staged linear mixed effects models. β (95% CIs) for change in annual eGFR slope per unit log$_2$-transformed plasma biomarker concentration within the subcohort (N=597).

Biomarker*	**Model 1** (eGFR)	**Model 2** (UPCR)	**Model 3** (eGFR + UPCR)
KIM-1	-0.77 (-0.96, -0.57)	-0.33 (-0.51, -0.14)	-0.34 (-0.54, -0.14)
TNFR-1	-0.97 (-1.38, -0.56)	-0.20 (-0.48, 0.08)	-0.43 (-0.81, -0.05)
TNFR-2	-0.98 (-1.46, -0.50)	-0.29 (-0.64, 0.05)	-0.53 (-0.97, -0.09)
MCP-1	-0.09 (-0.46, 0.28)	0.01 (-0.30, 0.33)	0.00 (-0.33, 0.33)
suPAR	-0.59 (-1.09, -0.09)	-0.20 (-0.56, 0.16)	-0.38 (-0.84, 0.08)
YKL-40	-0.63 (-0.87, -0.39)	-0.35 (-0.56, -0.15)	-0.39 (-0.61, -0.17)

KIM-1- kidney injury molecule-1; MCP-1: Monocyte chemotactic protein; TNFR-1: tumor necrosis factor 1; TNFR-2: tumor necrosis factor 2; suPAR: soluble urokinase-type plasminogen activator receptor; eGFR: estimated glomerular filtration rate (mL/min/1.73m^2); UPCR: urine-to-protein creatinine ratio
Base covariates for all models: age, sex, race/ethnicity, education, clinical center, systolic blood pressure, diastolic blood pressure, body mass index, hsCRP, hemoglobin A1c, anti-hypertensive medication use, smoking status
Model 1: base covariates plus baseline eGFR
Model 2: base covariates plus UPCR
Model 3: base covariates plus baseline eGFR and UPCR
*per log$_2$-transformed biomarkers

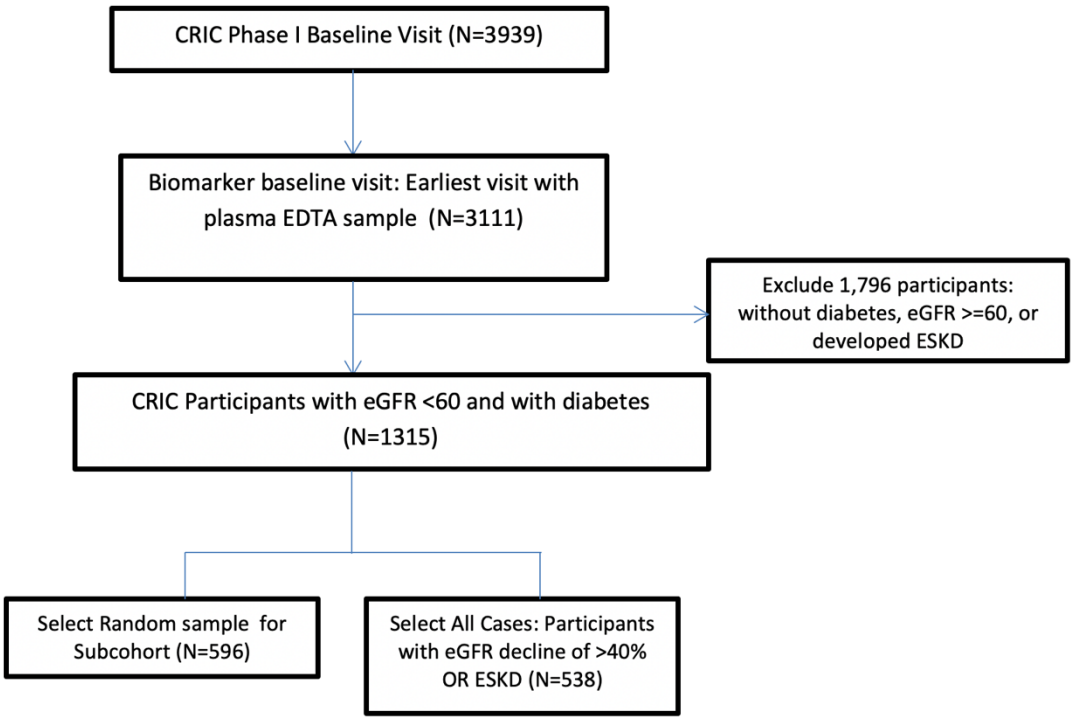

Figure S1: Flow Diagram of Study Sample Selection

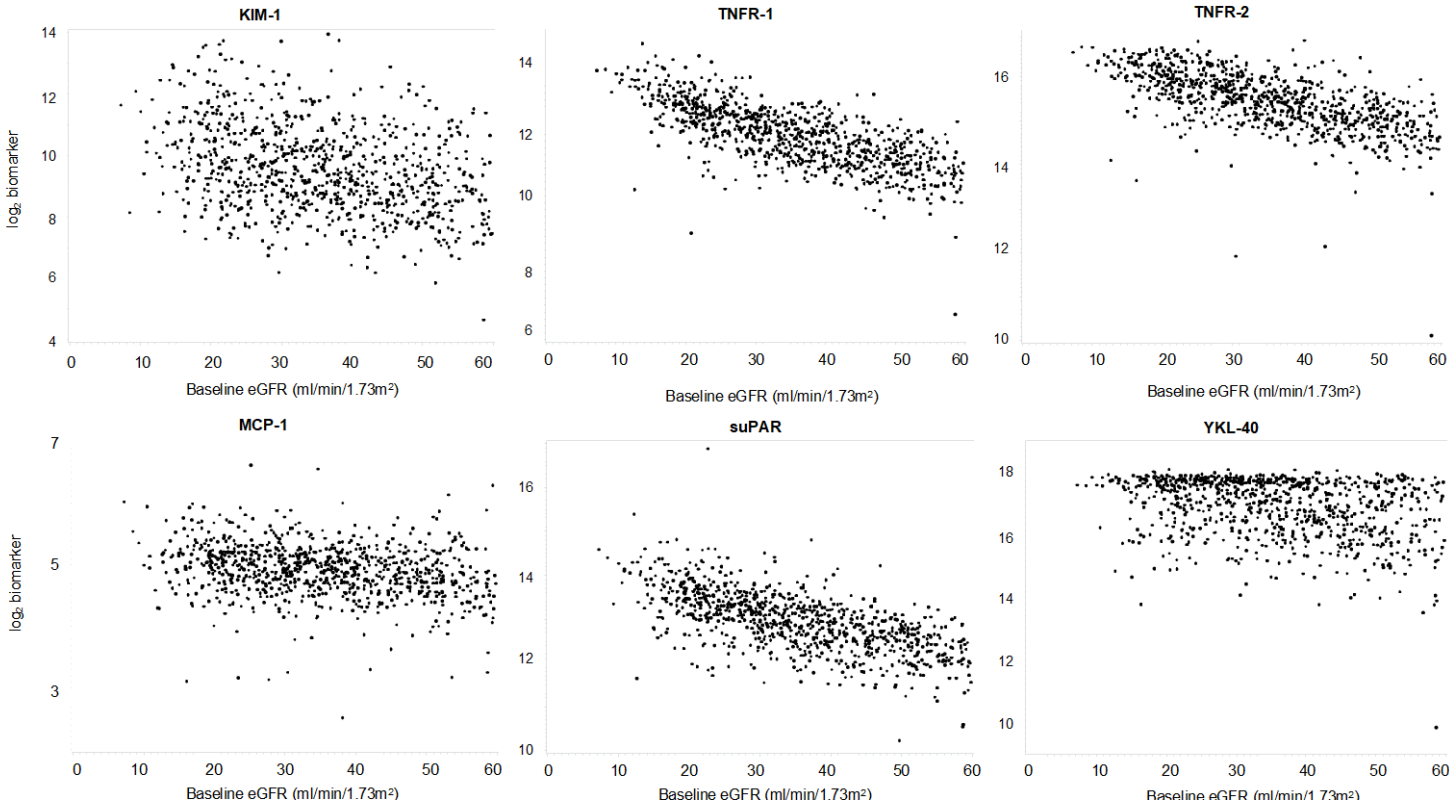

Figure S2: Scatterplot of plasma biomarkers (log_2-transformed in pg/ml) on y-axis by baseline eGFR (ml/min/1.73m^2) on x-axis. Top row (R-L): KIM-1, TNFR-1, TNFR-2. Bottom row (R-L): MCP-1, suPAR, YKL-40

Supplemental Methods for Evaluating Plasma Biomarkers for Prognostic Enrichment

The Biomarker Prognostic Enrichment Tool for Survival outcomes (BioPETsurv) is open source software found at: http://162.243.95.157/surv, which can assess the potential utility of plasma biomarkers to enrich clinical trial enrollment, and is similar to the previously published BioPET for binary outcomes, located at: http://162.243.95.157/orig.[1] BioPETsurv can simulate biomarker and time-to-event data that matches prespecified event rates with and without enrichment in terms of a hazard ratio. BioPETsurv displays Kaplan-Meier survival curves for the entire patient population and enriched subsets. Based on the level of enrichment, the prognostic strength of the biomarker, and length of the trial, BioPETsurv estimates the expected event rate absent intervention. The expected event rate with statistical testing specifications (e.g., power) and the treatment effect determine the trial sample size, which is dependent on the level of enrichment.

We set the BioPETsurv simulation parameters to reflect the range of hazard ratios for DKD progression that were observed to be associated with individual plasma $\log_2$ biomarkers in this study, from the most modest association of KIM-1 (HR 1.26) to the strongest association of TNFR-2 (HR 2.18). We specified constant hazards and normal distribution for the biomarkers. We set the survival data for 5,000 patients with event rate of 25% at 5 years, with 90% power to detect treatment hazard ratio 0.8 (two-sided testing, alpha=0.05), with conservative estimates for cost of screening of $100 per patient, and cost per-patient of $100/month to complete the trial.

The simulated results of KIM-1 are shown in **Figure S3**. Panel A shows estimated survival curves for screening threshold 0% (top curve), i.e., for all patients (no enrichment). The plot shows that events accumulate more quickly in enriched subpopulations of patients, showing more quickly decreasing survival curves for enrichment levels 25%, 50%, and 75% (meaning that patients with biomarker below the 25th, 50th, or 75th percentile are excluded). Panel B shows the estimated event rate increases as a function of the level of enrichment. Based on these event rates and specifying 90% power to detect treatment hazard ratio 0.8 (two-sided testing, alpha=0.05), panel C displays the sample size (decreases with greater enrichment). Panel D shows the number of patients needed to screen to enroll for the trial. With higher enrichment, the screening total increases. Panels E and F display the cost analysis, which shows cost

savings for higher levels of enrichment. **Figure S4** displays the simulated results of TNFR-2, the biomarker most strongly associated with DKD progression. **Table S8** displays the numeric results of the simulations.

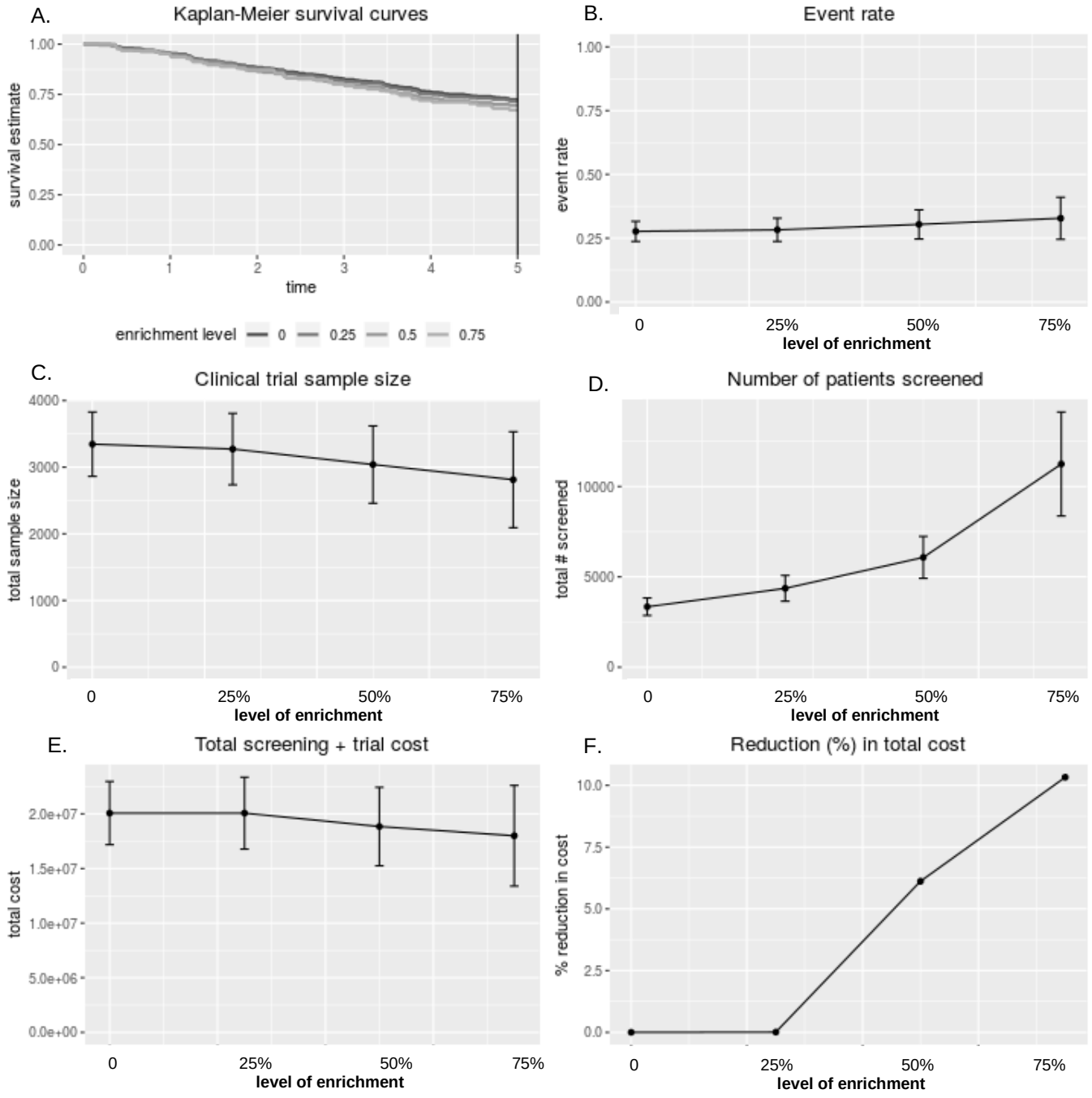

Figure S3: **BioPETsurv analysis of KIM-1 as a modest prognostic biomarker of DKD progression for a 5-year clinical trial.** The BioPETsurv data simulator generated data for a normally distributed biomarker with prognostic strength of HR 1.26 corresponding to change in $\log_2$ biomarker. Sample size calculations specified 90% power to detect a treatment hazard of 0.8 using two-sided testing and alpha=0.05. For cost analysis, patient screening was set at $100 and the cost of a patient in the trial was set at $100/month.

A.

B.

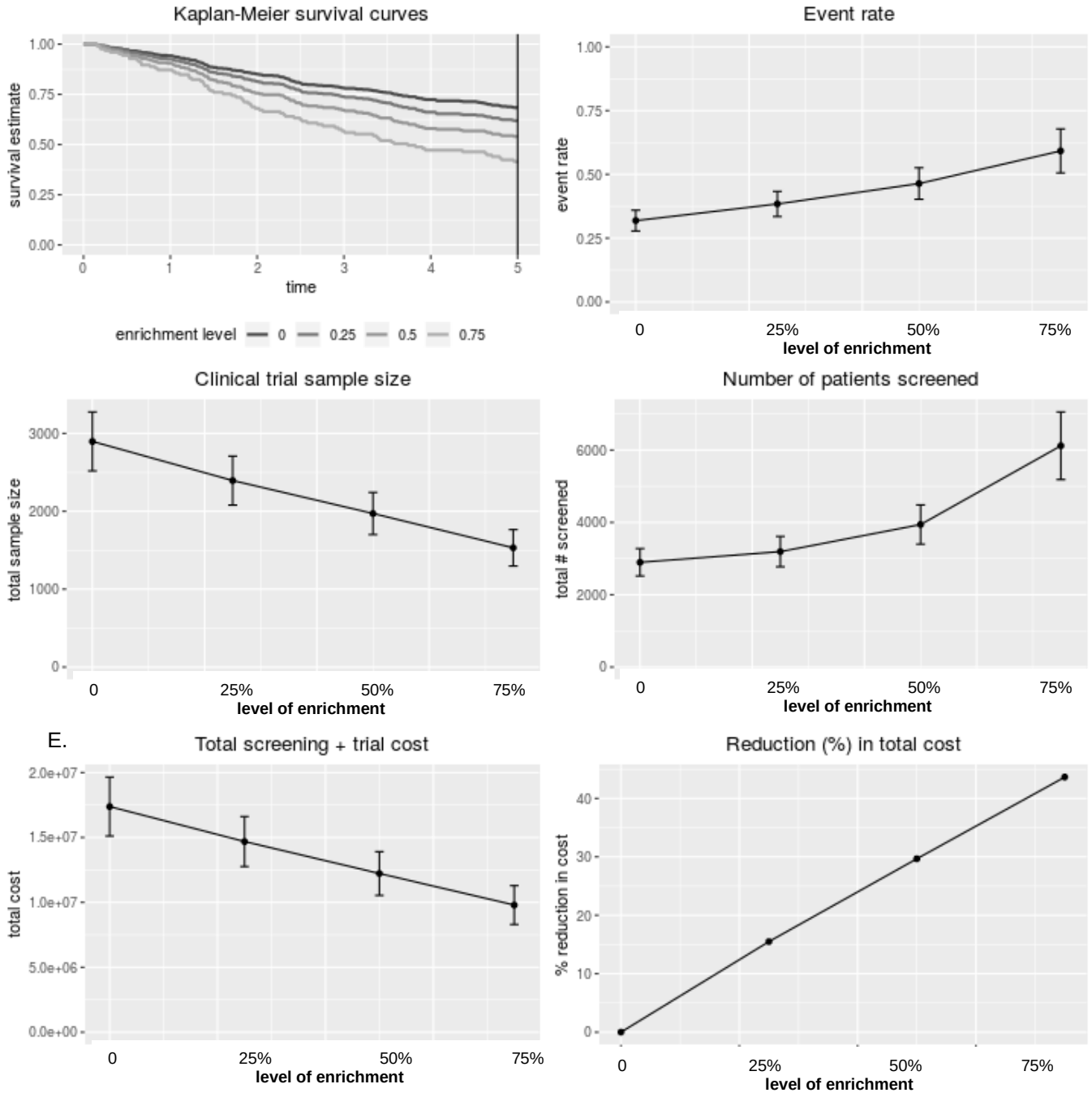

Figure S4: **BioPETsurv analysis of TNFR-2 as a highly prognostic biomarker of DKD progression for a 5-year clinical trial.** The BioPETsurv data simulator generated data for a normally distributed biomarker with prognostic strength of HR 2.18 corresponding to change in $\log_2$ biomarker. Sample size calculations specified 90% power to detect a treatment hazard of 0.8 using two-sided testing and alpha=0.05. For cost analysis, patient screening was set at $100 and the cost of a patient in the trial was set at $100/month.

Table S8: Simulation of modest to highly prognostic biomarkers of DKD progression for a clinical trial of 5 years.

KIM-1 (HR of 1.26 for DKD Progression)				
Screening Threshold (Level of Enrichment)	**Event Rate (%)**	**Sample Size**	**Total Screened**	**Reduction in Total Cost (%)**
0%	0.28	3345	3345	0
25%	0.28	3272	4363	0.01%
50%	0.30	3039	6078	6.12%
75%	0.33	2813	11,248	10.3%

TNFR-2 (HR of 2.18 for DKD Progression)				
Screening Threshold (Level of Enrichment)	**Event Rate (%)**	**Sample Size**	**Total Screened**	**Reduction in Total Cost (%)**
0%	0.32	2896	2896	0%
25%	0.38	2394	3192	15.5%
50%	0.46	1971	3942	29.67%
75%	0.59	1530	6120	43.65%

1. Kerr K, Roth J, Zhu K, et al. Evaluating biomarkers for prognostic enrichment of clinical trials. *Clin Trials.* 2017;14(6):629-638.